ATTENUATION OF EXERCISE-INDUCED BRONCHOSPASM

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I. INTRODUCTION

A. Asthma

The classification of asthma has long been debated because a clear understanding of this disease has eluded researchers. The simplistic dichatomous division of all asthma patients into extrinsic and intrinsic at first appears attractive (patients who develop wheezing when exposed to a specific allergen and who have an "atopic" history being labelled extrinsic asthmatics, and those who have no history of allergies and whose wheezing is usually precipitated by a respiratory tract infection being referred to as intrinsic asthmatics). However, as new and unique precipitants of asthma continue to be discovered, these simple definitions fail to give full insight into the mechanisms involved in this complex disease.

A more recent approach to understanding and classifying asthma has been to pursue those humoral and neural mechanisms felt to be common to all forms of asthma. This approach may prove particularly useful in designing better treatment regimens. In this study we chose to investigate the mechanisms of asthma by focusing on a particular stimulus of bronchospasm: exercise.

B. Exercise-Induced Bronchospasm (EIB)

As early as the 17th century, Willis[1] observed the correlation between exercise and asthma:

> "Whatsoever therefore makes the blood boyle or raises it into effervescence as violent motion of the body or mind, excess of extern cold or heat, the drinking of Wine, Vinery, yea sometimes meer heat of the bed doth cause asthmatical assaults to such as are predisposed."

Because exercise is a common, relatively predictable, and objectively measureable stimulus of asthma, researchers came to appreciate "the value of exercise as a diagnostic aid and a useful method for the assessment

of drugs in asthmatic subjects."[2] Therefore, EIB has now become a model

for the study of asthma in general. Despite the prevelance and importance

of exercise-induced bronchospasm, much remains unknown concerning its

mechanisms. Various theories of the triggering stimulus for EIB have been

proposed in the past, such as hypocapnia, increased minute ventilation,

acidosis, stimulation of receptors in the pharynx or carotid body, and airway

cooling. This confusing area has been partially elucidated by the development

of the "heat exchange" theory,[3] which correlates the total heat lost from

the bronchial airways during exercise and the subsequent bronchospasm.

Despite a clearer understanding of the factors responsible for the triggering

of EIB, the mechanism initiated by this stimulus remains far less clear.

Several mechanisms for EIB have been suggested, and convincing arguments

for and against all of them have been provided. Imbalance of the adrenergic

and cholinergic nervous systems has been proposed as a possible factor, and

studies have suggested that increased cholinergic tone,[4] β-adrenergic

blockade,[5] or an enhancement in α-adrenergic activity[6] may help explain the

etiology of EIB. However, it has been shown by other investigators that

adrenergic and cholinergic blockage do not change responses to exercise in

asthmatics.[7,8,9] Therefore, controversy exists concerning involvement of

the autonomic nervous system in EIB.

Various chemical mediators have been suggested as the primary etiologic

factor in exercise-induced asthma. This theory has largely been supported

by the fact that drugs such as sodium-cromoglycate, which inhibits mediator

release from mast cells, inhibit EIB if given before, but not after, exercise.[10]

A second argument that EIB is dependent on the release of mediators is that

the severity of attacks of EIB decrease when exercise is repeated after short intervals, suggesting release of a stored chemical mediator whose synthesis requires a certain amount of time.[11] Although evidence has been offered in favor of histamine,[12,13] prostaglandins,[14,15,16] slow-reacting substance of anaphylaxis (SRS-A),[17,18] and bradykinin,[19,20] the exact nature of the responsible mediator(s) remains unclear. Therefore, we decided to proceed with our study of the possible mechanisms of asthma by narrowing our focus to potential chemical mediators of EIB.

C. <u>Ascorbic Acid (Vitamin C)</u>

Although indirect evidence currently suggests that EIB is caused by release or formation of an agent with bronchoconstrictor activity,[21] direct evidence remains lacking, and investigators have continued in their endeavors to better define the "mediator(s)" responsible. One approach has been to search for substances that potentially inhibit or counteract a suspected mediator of EIB and to determine whether these agents have a protective effect against bronchospasm initiated by exercise. One such agent, ascorbic acid, has long been considered a potential inhibitor of asthma.

As early as 1938, H.B.Hunt stated that "there is a certain amount of evidence that ascorbic acid may play a part in allergic conditions in general and asthma in particular."[22] In addition, vitamin C has been found to have a role in the prevention of anaphylaxis and allergy[23,24] and in the modulation of sensitivity of smooth muscle to spasmogenic agents.[25,26,27] Zuskin was able to demonstrate an inhibition of bronchoconstriction by ascorbic acid by measuring the degree of bronchospasm induced in healthy subjects by histamine inhalation after either placebo or ascorbic acid.[28]

Although this finding has been challenged,[29,30] it does offer the possibility that vitamin C may have a anti-bronchospastic property.

Many theories have been advanced to explain the relationship between vitamin C and the reactivity of smooth muscle in general and bronchial smooth muscle in particular. A direct action of ascorbic acid on smooth muscle cells, perhaps mediated by a change in oxidation-reduction potential, has been suggested by some investigators,[27,31] while others stress the importance of vitamin C in histamine metabolism.[32] A third theory invokes fluctuations in prostaglandin levels (increase in PGE and/or decrease in PGF) induced by ascorbic acid.[33,34] Finally, alterations in levels of cyclic nucleotides provide a potential explanation for the mechanism of ascorbic acid's effect on airway dynamics.[35]

D. Present Work

Further elucidation of the mechanism by which the trigger of EIB leads to changes in airway resistance has practical and theoretical importance for a clearer understanding of EIB and asthma in general. The previously demonstrated anti-bronchospastic properties of vitamin C, as well as its involvement in the metabolism and activity of several potential mediators of EIB, suggested that investigation of this agent might prove fruitful. Since we believed that a chemical mediator etiology for EIB was correct, we hypothesized that ascorbic acid would offer protection to patients with EIB. Demonstration of a protective effect of vitamin C against EIB would not only lend support to the chemical mediator theory for the mechanism of EIB, but would direct attention toward certain specific members of the large group of mediators being considered.

Therefore, in a double-blind random fashion, we proposed to study

the protective effect of ascorbic acid vs. placebo against EIB in patients with documented, yet mild, exercise-induced asthma.

II. MATERIAL & METHODS

A. Subjects

Eleven asthmatic* subjects (4 male, 7 female) and ten healthy subjects (8 male, 2 female) without history of wheezing or asthma were recruited and informed consent was obtained. In order to select asthmatics with mild disease, members of the university and hospital community were chosen, avoiding the often more severe asthma of hospitalized and clinic patients. None of our subjects had been hospitalized for their asthma and none required corticosteroids. Each subject completed a detailed questionnaire concerning the presence of respiratory symptoms, allergies, and exercise-induced bronchospasm (EIB). The subjects' anthropometric data and responses to the questionnaire appear in Tables 1 and 3. All eleven asthmatic subjects complained of wheezing or dyspnea during or after exercise, while none of the ten healthy subjects had similar complaints.

B. Pulmonary Function

Using maximal and partial expiratory flow-volume curves (MEFV, PEFV) (see below), forced expiratory flow rates and volumes were measured in all subjects. A pneumotachograph-integrator system was used to obtain flow and volume, and the curves were recorded on a Brush 500 X-Y recorder (slew rate of 40 inches per second). After inspiring to 60-70% of vital capacity, the subjects exhaled forcefully to residual volume (creating the PEFV curve). They next immediately inspired to total lung capacity and performed a second forceful expiration to residual volume (creating the MEFV curve) (see Figure 2).

*asthma is a disease characterized by an increased responsiveness of the trachea and bronchi to various stimuli and manifested by a widespread narrowing of the airways that changes in severity either spontaneously or as a result of therapy (defined by American Thoracic Society)[36]

From these curves were obtained forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), peak expiratory flow rate (PEFR), and flow rates measured at 60% of the baseline vital capacity below total lung capacity on maximal and partial flow volume curves (MEF40% and MEF40%(P), respectively). In addition, the flow rate after expiration of 50% of the tidal volume following a maximal inspiration (VMax50%) was measured for the baseline curves. Baseline pulmonary function data for all subjects appears in Tables 2 and 4.

C. Exercise Testing

All subjects performed the exercise studies on a cycloergometer (Monark) and cardiac frequency was measured with an electrocardiograph (Hewlett-Packard). Baseline heart rate was obtained and exercise was begun at a constant speed of 20 kilometers per hour against zero workload. At the end of each one minute interval, cardiac frequency was measured and the workload was increased by 150 kilopond meters per minute, keeping the pedalling speed constant throughout the experiment. Continued exercise against progressively larger workloads was maintained until either the heart rate reached 170 or the subject fatigued.

D. Experimental Design

    a. Asthmatic subjects

        1. First Day

All eleven asthmatics were instructed to take no medications for at least eight hours prior to each phase of the experiment. They were similarly instructed to refrain from any food or beverage containing large amounts of methyl xanthines (coffee, tea, cocoa, soft drinks, etc.) or ascorbic acid

(citrus fruits, juices, etc.). On the initial screening day, baseline PFT's were obtained by having the subjects perform the partial and maximal flow volume maneuvers three times, at one minute intervals, generating three pairs of partial and maximal flow-volume curves (baseline curves). Exercise was then performed as described above. After completion of exercise, three new pairs of MEFV and PEFV curves were obtain ed at one minute intervals (immediate post-exercise curves). A third set of three pairs of curves was generated five minutes after termination of exercise (five-minute post-exercise curves). Two metered doses (0.65 mg each) of metaproterenol sulfate (Alupent[R]) inhaler were then administered, and ten minutes later three final pairs of MEFV and PEFV curves were obtained (post-alupent curves) (see Figure 1) FVC, FEV_1, PEFR, MEF40%, and MEF40%(P) values were determined for the four sets of curves by averaging the values obtained from the three pairs of MEFV and PEFV curves in each set. Those subjects who demonstrated sufficient EIB (20% reduction in MEF40% and MEF40%(P) after exercise) were allowed to proceed to the remaining phases of the experiment.

    2. Second and Third Days

    On the second and third days, each subject ingested (in double blind, random order) vitamin C (ascorbic acid 500mg) and placebo (sucrose) one hour prior to repeating the first day's experiment. In identical fashion, PFT's were measured before exercise, after exercise, and after alupent.

b. Healthy Subjects

Because our asthmatic subjects provided an internal control (taking vitamin C on one day and placebo on the other), control subjects were required only for the initial screening day without drugs to demonstrate that the asthmatics' response to exercise differed from that of normal subjects.

III. RESULTS

A. <u>Pulmonary Function Response to Exercise Without Drugs</u>

During the initial screening day for the asthmatics and the single experimental day for the healthy subjects, data measuring pulmonary function changes in response to exercise were collected. Neither group received vitamin C or placebo during this phase of the experiment in order to allow an unbiased determination of the presence or absence of EIB. Tables 5 through 9 display the changes in FVC, $FEV_{1.0}$, PEFR, MEF40% and MEF40% (P) after exercise for the asthmatic subjects, listing both the absolute quantities and the values as a percent of the before-exercise, or baseline, measurements (% baseline). In a similar fashion, Tables 10 through 14 represent the responses of the healthy subjects. Figures 5 through 9 graphically demonstrate these data. All changes from baseline were tested for statistical significance using the Student's paired t-test.[37]

In the asthmatic group, all five parameters demonstrated the typical response for patients with EIB: bronchodilitation during exercise (and therefore, immediately post-exercise), bronchoconstriction commencing within five minutes after exercise, and a marked bronchodilitation after inhalation of metaproterenol sulfate (post-alupent) (see Figures 3 and 4). The post-exercise bronchoconstriction is the most characteristic response to exercise in patients with EIB, and this change from baseline was statistically significant ($p < .01$) for all five parameters measured. Although none of the parameters revealed a statistically significant change from baseline immediately post-exercise, there was a consistent trend toward bronchodilitation. After administration of alupent, the bronchodilitation experienced by the asthmatics was statistically different from baseline ($p < .05$) for all parameters except FVC.

In contrast, healthy subjects revealed no post-exercise bronchoconstriction except for a small and statistically insignificant decrease in FVC. All other changes of pulmonary function in response to exercise

in non-asthmatics were not found to be significant when analyzed statistically except for an increase in MEF40% ($p < .05$) five minutes post-exercise (bronchodilitation) and increases after alupent in MEF40% ($p < .01$) and MEF40% (P) ($p < .01$).

Therefore, the asthmatic subjects as a group demonstrated significant bronchospasm post-exercise that was not observed in normal controls, confirming the diagnosis of EIB. Both groups revealed bronchodilitation in response to alupent (asthmatics > healthy subjects).

B. <u>Pulmonary Function Response to Exercise</u>: <u>Placebo</u>

Tables 15 through 22 display the measured change in pulmonary function with exercise after placebo and without drugs both as absolute quantities and as a percent change from baseline before exercise (" ΔPFT"). This information is represented graphically in figures 6 through 10. Tables 23 through 25 compare the differences in these ΔPFT's between placebo and without drugs (Δ (ΔPFT)). These differences between the ΔPFT's after placebo vs. without drugs was not within statistical significance for all five parameters. Therefore, no placebo effect was found. It is of interest that the changes in FVC, FEV_1, and PEFR demonstrated a trend of increased bronchospasm after exercise with placebo as opposed to the initial screening day. Similarly, there was less bronchodilitation in the asthmatic subjects immediately post-exercise and after alupent, as measured by all five variables, when exercise followed ingestion of a placebo as opposed to without drugs. However, this difference was not within statistical significance.

C. <u>Pulmonary Function Response to Exercise</u>: <u>Vitamin C</u>

Tables 26 through 33 display the change in PFT's with exercise following

ingestion of vitamin C vs. placebo both in the absolute and the ΔPFT form. This comparison is graphically portrayed in Figures 15 through 19. Tables 34-36 contain the $\Delta(\Delta$PFT) measurements for vitamin C vs. placebo. It is of note that ingestion of vitamin C, when compared to placebo, led to a lesser degree of bronchospasm after exercise as measured by all five parameters (statistically significant for FVC (p < .05) and $FEV_{1.0}$ (p < .05)). Similarly, vitamin C was associated with an increase in bronchodilitation relative to placebo both immediately after exercise and post-alupent as measured by all five variables. These differences between vitamin C and placebo were statistically significant for the immediate post-exercise measurements of PEFR (p < .05) and MEF40% (P) (p < .05) and for the post-alupent values of all parameters (p < .05) except MEF40%(P). Therefore, vitamin C, relative to placebo, was found to cause significant decrease in bronchospasm after exercise (for FVC and $FEV_{1.0}$) in patients with EIB. In addition, vitamin C led to a significant enhancement of broncho-dilitation both immediately post-exercise and after alupent as demonstrated by several of the parameters we used to measure pulmonary function.

Table 37 contains a summary of the paired t-test results for the comparisons of the efficacy of placebo vs. no drug and vitamin C vs. placebo.

In order to demonstrate the improvement in individual subjects' PFT's with AA, Figure 20 compares the change in FEV_1 with exercise for all twelve subjects after vitamin C and after placebo. Although there is some overlap of the response to exercise on the two experimental days, the measurements demonstrate the general tendency toward increased bronchospasm after placebo relative to vitamin C.

D. <u>Analysis of Variance</u>

In order to demonstrate that there was no variation in measurements
of baseline pulmonary function between the various experimental days, a one-
way analysis of variance was performed for the baseline FVC, $FEV_{1.0}$, PEFR,
MEF40%, MEF40%(P), and Vmax50% on the vitamin C, placebo, and no-drug days.
The results, which appear in Table 38, confirm that there was no difference
in pulmonary function before exercise between the three experimental days.

IV. LITERATURE REVIEW: ASTHMA

   A. History[38]

      Hippocrates (460-370 BC) made the first known reference to asthma
but did not discuss the disease in detail. Galen's understanding of this
disease was confused by his anatomical misconceptions. He believed that
conduits connected the brain and the respiratory tract. He considered
asthma to be a mechanical interference of respiration caused by the descent
of secretions from the brain into the respiratory tract. It was not until
the Renaissance that Galen's dogma was discarded and more precise observa-
tions and descriptions followed. Concepts of etiology, however, remained
confused. Laennec's treatise on auscultation in 1819 lead to great advances
in the understanding of all pulmonary disease. He suggested several preci-
pitating factors of asthmatic paroxysms but felt that the final common etiologic
event was a temporary alteration in nervous function. He believed that con-
traction of smooth muscle fibers surrounding the bronchi and air vessicles
provided a physiological explanation for the physical findings observed.

      Our understanding of asthma has obviously advanced greatly since
these beginnings as we have a far clearer picture of anatomy and pathophysiology.
However, the mechanisms responsible for producing the clinical symptoms have
remained a subject of debate, and this confusion is illustrated by the contro-
versies concerning the classification of asthma.

   B. Classification of Asthma

      Some researchers choose to separate all asthmatics into two large
groups: extrinsic or intrinsic. Extrinsic bronchial asthma, or allergic
asthma, is a disease predominantly of children and young adults. Episodes

of bronchospasm are short-lived, interspersed between symptom-free intervals, and temporally related to exposure to certain causitive antigens. These patients often have an "atopic" history, demonstrating other allergic phenomena such as eczema or hay fever. Allergic asthmatics generally respond well to medical treatment, but they may have recurrences in later adult life.

Intrinsic asthmatics are a more heterogeneous group of patients in whom antigenic exposure does not appear to play a role in producing broncho-spasm. These patients tend to be adults over thirty-five years of age, and they usually have no history of allergies. Because respiratory tract in-fections seem to be important in the development of symptoms, this form of asthma has also been referred to as infective asthma. Aside from increased cough and sputum production, these bronchospastic episodes closely resemble those of allergic asthmatics. Infective asthma is more likely to be a chronic problem that is less responsive to medical management.

Despite the obvious convenience of creating the categories of ex-trinsic and intrinsic asthma, this strict dichotomy breaks down with closer analysis. Many patients are labeled mixed asthmatics, demonstrating character-istics of both groups; they are atopic and have an allergic basis to their bronchospasm, but their acute episodes are usually preceded by a respiratory tract infection. The concept that a large group of asthmatics develop symptoms on a non-allergic basis has itself been challenged, and some have argued that immunological factors are involved in all forms of asthma even though they cannot be demonstrated. Others have proposed that the cases of bronchospasm in which immunological involvement cannot be implicated should not be con-sidered asthma.

Further problems with this simplistic dichotomy become evident on more careful scrutiny of asthmatic patients. Many have been classified into minor categories of asthma, but these groups tend to cross the artificial boundary between intrinsic and extrinsic asthmatics. One such group, exercise-induced asthma, contains a diverse population of asthmatics from those with a strong history of atopy to others who have classic intrinsic bronchospasm.

There are other subtypes of asthma that create similar problems in classification.[39] Patients with "asthmatic-bronchitis" may be thought of as having chronic bronchitis with superimposed symptoms of asthma without a clearly defined allergic basis. A small number of patients with intrinsic asthma manifest the triad of asthma, aspirin sensitivity, and nasal polyposis. Their bronchospasm is usually preceded by aspirin ingestion. A final group of patients, dual Type I and Type III reactors, develop an acute attack of bronchospasm (IgE-mediated) within ten to fifteen minutes after exposure to an allergen and then a second (IgG-mediated) response in two to six hours.

Confused by these overlapping artificial categories, some researchers began to consider asthmatic patients as a spectrum from those with a strong history of allergic stimulation to those with no known immunologic basis for their bronchospasm. Although each patient is particularly susceptible to certain stimuli, there may be some precipitants of asthma that provoke an attack in all patients. Therefore, what is needed for the study of asthma is a suitable experimental human model in which acute exacerbations can be induced.

V. LITERATURE REVIEW: EIB

A. History

After Willis first recognized in 1684 that asthma could be stimulated by exercise (or "whatsoever...makes the blood boyle"), Floyer recorded in 1717 an attack of asthma precipitated by exercise and stated that "all violent exercise makes the asthmatic to breathe short."[40] He further noted that different types of exercise varied in their ability to provoke wheezing.

Very few references were made to this correlation between exercise and asthma throughout the eighteenth and nineteenth centuries. In 1946 Herxheimer measured the change in vital capacity after exercise in asthmatic subjects,[41] performing the first objective study of the relationship of asthma to exercise. He concluded that hyperventilation was the cause of this broncho-spasm stimulated by exercise.

Herxheimer's work stimulated many researchers interested in this field, but there was much confusion due to conflicting reports of either increased bronchospasm or improvement of pulmonary function after exercise. Jones et al in 1962 first attempted to explain this paradox and studied the response of asthmatic children to running exercise for brief (two minute) and prolonged (eight to twelve minutes) periods. They found that the FEV_1 increased after the former but fell markedly after longer durations of exercise.[42] Although his findings were not supported totally by later work, he did demonstrate the importance of the duration of exercise.

Further work in this field suffered from several misconceptions concerning the stimulus for EIB. Various conflicting theories for the triggering stimulus were proposed, and different forms of exercise were felt

to have varying ability in promoting bronchospasm. It was not until recently, when McFadden et al developed the heat exchange theory for the trigger of EIB (see below), that much of the conflicting data could be explained and a clearer understanding of this disease became possible.

B. EIB as a Model for Asthma

Jones et al first suggested that EIB might be used as a diagnostic test for asthma in children based on his demonstration that nineteen of twenty-one asthmatic children developed bronchospasm (measuring FEV_1) after eight to twelve minutes of running.[43] Much research followed to confirm that exercise was a reliable stimulus for bronchospasm in asthmatics. Godfrey demonstrated that ninety percent of asthmatic children had a ten percent decrease in PEFR after exercise[44] whereas this response is rarely seen in healthy children.[45] Using a fifteen to twenty percent decrement in FEV_1 as a criterion, others have demonstrated a sixty to seventy percent incidence of post-exertional asthma among children.[46,47]

Similar results have been observed in adults. McNeill et al demonstrated a thirty to seventy-five percent decrease in FEV_1 after exercise in all ten adolescents and young adults he studied.[48] A meticulous study by Haynes, Ingram, and McFadden measured airway resistance (using a constant volume plethysmograph), airway specific conductance, FEV_1, flow rates between twenty-five and seventy-five percent of FVC (MMFR), and maximal flow rates at isovolumes between forty and sixty percent of TLC ($\dot{V}$ max iso) in twenty-one asthmatics and eight normal controls. They found that all of the asthmatics, in contrast to the controls, had a significant response that was measureable by at least one variable irregardless of the severity of their clinical history.[49]

Finally, the development of the PEFV curve,combined with instantaneous measurements of flow rates at low lung volumes,provided evidence that exercise was even more of a ubiquitous stimulus for bronchospasm than had previously been comprehended (see below).[50]

Therefore, the ability of exercise to stimulate an attack of bronchospasm in most, if not all, asthmatics has led investigators to consider EIB as a suitable model for the diagnosis and study of asthma[51,52,53,54] Jones has stated that "a post-exercise fall in FEV_1 is so constant in the asthmatic that a failure to demonstrate it should lead to reconsideration of the diagnosis or of the technique of the test."[42] Some have suggested that EIB may not be an isolated disease, but rather that exercise is just one means of stimulating bronchospasm in asthmatics.[55]

Exercise has been found to have several advantages compared to other methods of evoking an acute asthmatic response. Unlike the inhalation of pharmacologic or immunologic agents, exercise avoids direct stimulation of the airway itself and artifactual alterations in pulmonary function.[49,51] In addition, exercise produces a response in atopic as well as non-atopic asthmatics.[56] Finally, exercise can be used in the laboratory to safely and reliably induce exacerbations of bronchospasm.[49]

C. Response to Exercise in Asthmatics and Healthy Subjects

The most accurate and sensitive parameters for measuring the response of asthmatics to exercise has long been the subject of debate. Researchers have commonly used FEV_1,[42] PEFR,[57] airway resistance,[49] maximal mid-expiratory flow rates (MMFR),[49] total lung capacity (TLC),[53] and FVC[53] to detect changes in pulmonary function. The typical response of asthmatics to five to

ten minutes of exercise is an improvement in pulmonary dynamics during exercise and a marked decrease commencing three to five minutes afterwards.[2] The response of healthy subjects has been reported to be either a statistically insignificant change from baseline[43] or else markedly less than the change observed in asthmatics.[2]

The measured improvement in pulmonary function during exercise in asthmatics, which has been attributed to the release of endogenous catecholamines,[55] has been exploited in attempts to develop more sensitive measures of the response to exercise. Jones suggested a "bronchial lability index" $\frac{\text{Fall of FEV}_1 + \text{Rise of FEV}_1}{\text{Predicted Normal FEV}_1 \text{ at rest}} \times 100$, where "Fall in FEV_1" is the maximal decrease after five to eight minutes of exercise, and "Rise in FEV_1" is the maximal bronchodilitation after isopenaline aerosol and one minute's exercise. Studying asthmatic and healthy children, he demonstrated that, with few exceptions, the lability index was greater than twenty percent in asthmatics and less than fifteen percent in control subjects.[58]

Bouhuys questioned the traditional use of the MEFV curve to assess subtle changes of induced bronchoconstriction because it had been demonstrated previously that a deep inspiration might lead to a transient increase in airway resistance.[59,60] He demonstrated that flow rates on PEFV curves, where the maneuver is initiated by a less than complete inspiration, were more sensitive in detecting bronchoconstriction than flow rates on MEFV curves at comparable volumes.[61] He postulated that this might reflect inhibition of bronchoconstriction that is usually produced by the deep inspiration involved in the MEFV maneuver and potentially obscures subtle mechanical changes. He further criticized the use of standard measurements of pulmonary

function (FEV_1, MMFR, PEFR, etc.) because they are partially a function
of the effort-dependent (early) part of the FVC. He advocated the use of
instantaneous flow rates at lower lung volumes (MEF40%, MEF40% (P))[62],
using electronic circuits to record flow and volume[63], in order to more
accurately measure the state of the small airways during the effort-
independent (late) portion of the FVC. These techniques led to increased
sensitivity in measuring bronchospasm initiated by exercise in asthmatics[50]
and by pharmacologic agents in healthy subjects.[64]

    D. Trigger of EIB

       1. Early concepts

          In attempting to elucidate the "trigger" that is activated
by exercise, researchers have accumulated conflicting data. Furthermore,
different forms of exercise have been shown to result in varying degrees
of bronchospasm, a finding that has generated added debate.

          a. _Hypocapnia_ has long been considered a potential cause of
bronchoconstriction. Hafez and Crompton[65] demonstrated a small but
statistically significant decrease in FEV_1 in asthmatics after hyperventila-
tion that was not observed in bronchitics. Furthermore, there was no change
in pulmonary function after hyperventilation induced by carbon dioxide
inhalation. Furguson _et_ _al_ measured P CO_2 levels after exercise in healthy
controls and in patients with EIB and diffuse lung disease. Three minutes
after exercise, the P CO_2 of the asthmatics was 23 mm Hg compared with 37
for controls and 31 for patients with diffuse lung disease. They postulated
that the hypocapnia produced bronchospasm because it could be reversed by
adding carbon dioxide to the inspired air.[66] In addition, Fisher _et_ _al_

demonstrated that bronchospasm stimulated by running could be reversed
by inhaling six to eight percent carbon dioxide but not by inhaling
atropine sulfate, suggesting an effect of carbon dioxide on smooth muscle
unrelated to a vagal efferent reflex pathway.[54]

b.   Others have claimed that mechanical _hyperventilation_ itself,
and not hypocapnia, is the inciting stimulus for EIB. Chan-Yeung
demonstrated bronchoconstriction in asthmatic patients despite normal
or slightly increased concentrations of carbon dioxide in airways and
blood.[9]

c.   A third popular theory for the trigger mechanism is the release
of _lactic acid_ from skeletal muscle cells during the anaerobic phase of
exercise. Many researchers have correlated bronchospasm with the presence
of metabolic acidosis and/or increased circulating levels of lactate after
exercise in patients with EIB.[9,54,67] Schiffman _et al_ postulated that this
change in lactic acid concentration might be causing bronchospasm by
stimulating the carotid bodies, and he demonstrated that breathing one
hundred percent oxygen attenuated EIB in patients with intact carotid bodies
but had no effect in asthmatics who had undergone bilateral carotid body
resections.[68] Other suggested pathways for lactate's effect include release
of mediators from mast cells or increased production of bradykinin caused
by elevated hydrogen ion concentration.[3]

2.   Respiratory Heat Exchange

Different responses have been noted in asthmatics when exercise
has been accompanied by nasal breathing as opposed to oral breathing.
Souhrada _et al_ postulated that this might reflect the presence of receptors

in the oropharynx sensitive to temperature or humidity. These receptors
would be stimulated by exposure to cold, dry air breathed through the
mouth but not by air that had been "conditioned" as it was warmed and
humidified during its passage through the nasal airways.[69] Further
evidence for this theory was provided by studies demonstrating that EIB
can be attenuated by oral anaesthetics, suggesting that EIB might be
triggered by a reflex mechanism initiated by stimulation of these irritant
receptors.[70]

The specific type of exercise used to elicit EIB has long been
known to be an important determinant of the degree of bronchospasm produced.
Fitch and Morton showed that EIB could be induced in forty asthmatic subjects
after eight minutes of exercise in 72.5 percent of running tests, 65 percent
of cycling tests, and 35 percent of swimming tests.[71] Others have agreed
that running is a more potent stimulus than cycling, and that walking and
swimming have variable effects.[72]

Much of the conflicting data presented above was put into perspec-
tive by a series of ingenious experiments by McFadden et al. Addressing
first the question of different responses to different forms of exercise,
they demonstrated that airway obstruction was dependent on the amount of
stress of participating muscles rather than on the total amount of external
work.[73] Larger workloads in relation to muscle mass stimulated higher
minute ventilation, higher hydrogen ion concentration, lower end-tidal
carbon dioxide tension, and increased bronchospasm.

Approaching the effects of _hyperventilation_ and _hypocapnia_,
they simulated minute ventilations achieved during exercise using a partial
rebreathing technique with variable end-tidal carbon dioxide tensions.

They found that even the most marked changes induced by hyperventilation or hypocapnia were small relative to bronchospasm produced by exercise at higher end-tidal carbon dioxide levels. This led to the conclusion that hypocapnia and the mechanical effects of bulk air flow were not the stimulus for EIB.[74]

The effect of lactic acid and hydrogen ion concentration were then addressed. Patients with EIB were exercised until bronchospasm was achieved. This experiment was repeated with sodium bicarbonate infusions to maintain baseline arterial pH. No change in the degree of bronchospasm was observed. Resting subjects with EIB received infusions of lactic acid at levels higher than those produced endogenously during exercise with no measureable change in pulmonary function. They concluded from these experiments that neither pH nor lactate levels played a key role in triggering bronchospasm.[75]

McFadden et al then observed that asthmatic subjects had greater decreases in pulmonary function when they exercised in a cold environment.[76] Because atropine did not decrease cold air's stimulus to bronchospasm, stimulation of sensory nerve endings and vagal reflexes did not appear to play a role.[77] Esophageal temperatures at the level of the mid-trachea were shown to be decreased relative to behind the heart and in the rectum when exercise was performed in room air, and this change in temperature could be increased by breathing colder and drier air.[78] Therefore, it appeared that bronchoconstriction was due to a local effect of incompletely conditioned air reaching the lower respiratory tract.

As a final series of experiments, McFadden et al studied the effects of heat and humidity on EIB by exercising asthmatics breathing, in random

order, air at four conditions: ambient room temperature and humidity, body temperature and ambient humidity, ambient temperature and one hundred percent humidity, and body temperature and one hundred percent humidity. Asthmatics breathing ambient air had typical bronchospasm, and heating the air led to no improvement. Some reduction in airway obstruction was achieved by humidifying the air, while saturated air at body temperature prevented EIB.[79] McFadden et al suggested that asthmatics may not be effectively conditioning air or may be unusually sensitive to airway cooling.[3]

The heat-flux hypothesis, the endpoint of this series of logical experiments, states that there is an association between the heat lost from the airways and the subsequent change in pulmonary function.[80] Air is warmed as it passes the respiratory tract mucosa moving from the environment to the lungs. As the temperature increases, the capacity of the air to hold moisture is enhanced and it is humidified by evaporation from the airway mucosa. This evaporation accounts for a large portion of the airway heat loss. Therefore, the higher the rate of ventilation or the colder and drier the air, the greater the heat exchange.[3] To test this theory, McFadden et al had resting subjects with EIB inhale air of various temperatures and humidities to simulate the "thermal burden" of exercise.[81] As expected, increased ventilation and decreased temperature or saturation of the air led to larger decrements in FEV_1. By plotting the percent change in FEV_1 (% ΔFEV_1) against the respiratory heat exchange (RHE) in Kcal/minute, the authors demonstrated a linear relationship between these two parameters: % ΔFEV_1 = 26.14 RHE - 4.16.[3]

E. Theories of the Mechanism of EIB (Beyond the Trigger)

Potential mechanisms of EIB, paralleling those of asthma in general, have stressed both chemical mediators and involvement of the autonomic nervous system.

1. Autonomic Nervous System (ANS)

Although a chemical mediator theory for EIB has received much attention recently, some still advocate ANS imbalance as a potential factor. The arguments proposed can be assigned to three main theories.

a. β-adrenergic Theory

β-adrenergic dysfunction has long been suggested as a mechanism of bronchoconstriction in asthmatics. Stimulation of β-adrenergic receptors was shown to cause bronchodilitation by a direct effect on smooth muscle cells. This response has been associated with increases in cyclic AMP (cAMP) levels. Szentivanyi summarized previous evidence and formulated the β-adrenergic theory which stated that the mechanism for bronchospasm in asthmatics might be defective β-adrenergic receptors, possibly secondary to an enzymatic defect in adenylate cyclase.[82] Parker and Smith demonstrated that leukocytes from asthmatics had a significant decrease in their cAMP response to β-adrenergic agents, suggesting some form of β-blockade.[83] This theory has been supported by the demonstration that pharmacologic β-blockade increases bronchospasm caused by allergens[84] or methacholine.[85] In addition, drugs which prevent breakdown of cAMP by inhibiting phosphodiesterase (i.e. methylxanthines) help to relieve asthma.[86]

β-adrenergic dysfunction has also been suggested in EIB. Jones demonstrated increased bronchial lability after exercise in five of six asthmatics and in seven of forty-five controls with mild β-blockade induced by propranolol.[87] Patel et al suggested that β-blockade leads to increased α-adrenergic response to catecholamines released during exercise.[5] Beil et al found that the increase in bronchospasm experienced by patients with EIB after propranolol could be inhibited by α-adrenergic blockade with phentolamine.[6] Although β-sympathomimetics have been shown to improve symptoms of EIB, this cannot be rigorously assigned to a direct

smooth muscle effect, as they also prevent mast cell degranulation.
In addition, there is evidence that sympathomimetics' prevention of
post-exertional bronchospasm may be distinct from their bronchodilating
effect.[88]

b. α-Adrenergic Theory

Bianco et al demonstrated the presence of α-adrenergic
receptors in bronchial smooth muscle cells and observed that indoramin,
an α-adrenergic blocking agent, was able to prevent EIB as measured by
FEV_1 and airway conductance. He postulated that, after exercise, endo-
genously released catecholamines stimulate the α-receptors which may be
either too numerous or too sensitive relative to the β-receptors in
patients with EIB.[89] However, Seale et al repeated this work using PEFR
to evaluate pulmonary function and found no attenuation of bronchospasm.[90]
In addition, indoramin has been shown to have an antihistaminic effect
which could account for its protective effect.[89]

c. Cholinergic Theory

Parasympathetic reflexes have been shown to play an important
role in airway dynamics. One example is the vagally mediated cough or
"irritant" receptor reflex.[4] These receptors can be stimulated by cough,
rapid inspiration or expiration, histamine, citric acid, chemically inert
dusts, or sulfur dioxide. In addition, it has been shown that immunologically
induced mediator release can be stimulated by parasympathomimetic drugs.[91]

Kiviloog implicated vagal involvement in EIB when he found
a positive correlation between post-exercise bronchospasm and methacholine
sensitivity. He concluded that bronchoconstriction by both stimuli worked

through nonspecific bronchial hyperreactivity.[92] Crompton reported a case of EIB that was prevented by atropine, suggesting that vagal reflexes might be involved.[93] Evidence to the contrary is provided by an experiment by Fischer et al in which atropine did not prevent EIB in seven asthmatic subjects.[54] Some researchers have suggested that cholinergic pathways cause bronchospasm in EIB when obstruction is limited to large airways, while mediators are involved in patients with more widespread bronchoconstriction.[94,95] McFadden et al has postulated that cholinergic reflexes themselves may be stimulated by mediator release.[95]

Sey et al performed an experiment which suggested that there is no ANS involvement in EIB. He measured the change in PEFR induced by exercise in ten asthmatic children after either α-adrenergic, β-adrenergic, or choliner-gic blockade and found no change in the post-exercise decrement in pulmonary function.[7]

2. Mediators

The release of histamine and other chemical mediators from mast cells in antigen-induced, IgE-mediated asthma has stimulated researchers to look for a similar mechanism for EIB. Sodium cromoglycate is a drug which stabilizes mast cells by preventing cAMP breakdown. It has also been found to have phosphodiesterose inhibitory capacity. Silverman et al demonstrated that this agent inhibits EIB if given before, but not after, exercise, suggesting that release of pre-formed, stored mediators are involved.[10] The protective effect of this drug has been confirmed by others.[96,97,98,99,100,101] The presence of a refractory period when broncho-spasm is repeatedly induced by exercise provides a second argument for the

mediator hypothesis.[9,48,102]

   a. Histamine

          The release of histamine is closely linked to the β-adrenergic
system. As is the case in smooth muscle cells, β-adrenergic stimulation
leads to an increase in cAMP in mast cells which stabilizes their membranes
and hence prevents degranulation. Evidence for histamine's involvement
in asthma independent of the β-adrenergic nervous system stems from the
demonstration that allergens, combining with IgE molecules on mast cell
membranes, lead to degranulation and histamine release.[103] Furthermore,
inhalation of histamine aerosol has been shown to produce bronchospasm in
asthmatics, although some have suggested that this may represent stimulation
of irritant receptors in patients with hyperactive airways. Nevertheless,
subcutaneous histamine causes bronchospasm in asthmatics with doses smaller
than those necessary to produce similar symptoms in healthy subjects.[104]
These effects of histamine in asthmatics have lead to suggestions that
histamine may play a central role in EIB.

          Anderson et al demonstrated that B-sympathomimetics attenuate
EIB through means other than bronchodilitation.[88] Zielinski et al found
that thiazinamium, an antihistaminic agent, prevented EIB and suggested that
prevention of histamine release might be another effect of β-adrenergic
stimulation.[105] More direct evidence for involvement of histamine in post-
exertional asthma was provided when Anderson et al demonstrated that arterial
plasma histamine levels increased during exercise in patients with EIB, and
this rise was diminished by β-sympathomimetics.[106] This finding contradicted
earlier work demonstrating no increase in histamine turnover during exercise
in these patients.[107]

b.   Slow-Reacting Substance of Anaphylaxis (SRS-A)

The chemical structure of this acidic lipid has not been
completely characterized. As opposed to the brisk contraction of
in vitro smooth muscle preparations induced by histamine, this substance
causes a more gradual contraction. In addition, relaxation is a slower
process when SRS-A is washed from the tissue baths as compared to other
stimulants of muscle contraction. SRS-A does not appear to be stored in
a preformed state in the tissues of man or the rat, and antigen-antibody re-
actions have been shown to be required for its release.[108]

Involvement of SRS-A in asthma has been postulated as this
chemical can be recovered from lung tissue that has been sensitized with
IgE and then immunologically challenged. Because its level rises in
concert with histamine, it is felt that SRS-A is also released from mast
cells. In addition, SRS-A has been shown to potentiate the effect of
histamine on smooth muscle.[108]

Indirect evidence has been provided for involvement of SRS-A
in EIB. Orange et al demonstrated that diethylcarbamazine citrate inhibits
release of SRS-A in rats,[109] and Sly et al subsequently showed that this substance,
given before exercise, prevented EIB in fifteen of twenty asthmatic children.[110]

c. Bradykinin

Kinins have been implicated in vasodilation, increased vascular
permeability, smooth muscle contraction, pain, and possibly leukocyte margina-
tion, yet their mechanism of action remains unknown.[111]

Kinins cause contraction of uterine and guinea pig ileal muscle
in vitro. Because this effect cannot be prevented by atropine, cholinesterase

inhibitors, or ganglionic blocking agents, it is postulated that kinins have a direct action on smooth muscle. This effect on certain types of smooth muscle, including bronchial, is variable, causing relaxation in some systems and contraction in others.[111]

The involvement of bradykinin in asthma was suggested by Collier et al with the demonstration that this agent produced bronchospasm in anesthetized guinea pigs and in isolated, perfused guinea pig lungs.[112] Herxheimer et al reproduced this work in guinea pigs and further showed that inhalation of bradykinin aerosol caused a decrease in vital capacity in asthmatics but not in healthy subjects.[113] However, no experiments have been carried out to study the involvement of bradykinin in EIB.

d. Prostaglandins (PG)

Prostaglandins are twenty-carbon, unsaturated fatty acids composed of a cyclopentane ring and two aliphatic side chains. Although there are four main groups of PG's, PGE_2 and $PGF_2\alpha$ have received the most attention in pulmonary medicine. Both are synthesized from arachidonic acid by prostaglandin synthetase. $PGF_2\alpha$ is formed by reduction of the intermediate form, cyclic endoperoxide, whereas isomerization leads to production of PGE_2.

Although PG's are found in most mammalian tissues, the lung has one of the highest concentrations.[114] Lung parenchyma contains ten to twenty times more $PGF_2\alpha$ than PGE_2, while two to three times more PGE_2 than $PGF_2\alpha$ can be found in isolated bronchi.[114] PGE's have a bronchodilating effect in isolated human bronchial muscle which is not altered by atropine or antihistamines.[115] $PGF_2\alpha$ causes bronchoconstriction that is not modified by SRS-A, α - and β-adrenergic agents, or serotonin.[114] Therefore, it appears that PG's influence bronchial smooth muscle tone independently of the ANS or other chemical mediators.

Some have suggested that PG's act directly on smooth muscle by raising cAMP levels. In human lung fragments and peripheral lymphocytes, PGE has been shown to increase cAMP concentrations by stimulating adenylate cyclase. This effect is not modified by β-adrenergic blocking agents. $PGF_2\alpha$ has been shown to have less or no cAMP stimulating activity relative to PGE_2. In addition, $PGF_2\alpha$ has been shown, in other tissues, to raise levels of cyclic GMP, a known antagonist of cAMP.[114]

There has also been indirect evidence implicating an effect of PG's on the calcium-dependent phase of smooth muscle contraction. PG's have been shown to alter the membrane permeability of calcium in smooth muscle preparations.[116] The potency of PG's to affect smooth muscle's contractile state has been related to the calcium concentration in the surrounding medium.[114] Carsten has provided evidence that PG's stimulate muscle contraction by increasing calcium release from smooth muscle sarcoplasmic reticulum.[117]

Recent evidence has suggested that PG's may play a role in bronchial asthma and EIB. Mathe et al demonstrated that aerosol $PGF_2\alpha$ causes bronchoconstriction in both asthmatic and healthy subjects, but asthmatics hyperrespond.[118] They suggested that bronchoconstriction in asthmatics may be due to local effects of $PGF_2\alpha$. Parker and Synder, however, have suggested that this may only be a response of irritant receptors to the aerosol propellant.[119] Allegra et al showed that the baseline peripheral plasma PGE levels in asthmatics is significantly higher than in nonasthmatics.[120] They postulated that this is a compensatory mechanism in asthmatics to raise cAMP levels in bronchial smooth muscle cells (possibly to overcome lower baseline

cAMP levels due to decreased β-adrenergic responsiveness). Later work by this same laboratory, however, failed to demonstrate changes in peripheral PG levels after exercise in patients with EIB, but these measurements did not reflect local bronchial changes in PG concentrations.[121] Indirect evidence against a role for PG's in asthma and EIB include the inability of sodium cromoglycate to prevent $PGF_2\alpha$ - induced bronchoconstriction[122] and the failure of indomethicin, an inhibitor of prostaglandin synthetase, to prevent EIB at a dose of two hundred milligrams daily.[123]

VI. LITERATURE REVIEW: ASCORBIC ACID

A. Background

Lind demonstrated in 1757 that an essential dietary factor caused scurvy, a common disease of seamen of his day.[124] He showed that fresh vegetables and citrus fruits prevented this disorder. Holst and Frolich induced scurvy in guinea pigs, and this experimental model aided Szent Gyorgy, an ingenious Hungarian researcher, to isolate ascorbic acid and to determine that it is a single crystalline substance. Reisseissen, in the early nineteenth century, first described the symptoms of "convulsive asthma" in severely scorbutic patients,[125] suggesting that vitamin C might somehow prevent symptoms of asthma.

Ascorbic acid is synthesized from glucose by many species of plants and animals via the glucuronic acid pathway. The ability to synthesize this molecule is absent in invertebrates, insects, and fish. Amphibians gained the synthetic capacity in their kidneys, and this ability was transferred to the reptiles and the lower orders of birds. The site of vitamin C biosynthesis was transferred to the liver of mammals and the more evolved birds. Finally, the capacity to produce this molecule was lost by the guinea pig, the flying mammals, the monkey, man, and a number of highly evolved passeriformes birds.[126,127] Some have suggested that the ability to synthesize this chemical was developed by amphibians as they entered terrestial life because ascorbic acid functioned in some manner to overcome the more stressful conditions of life on land.[126] The single gene mutation postulated for the loss of this biosynthetic capacity might not have been lethal as ascorbic acid was present in the diets of the mutant animals.[126]

H. The Relation of the Biochemical Effect of Vitamin C to
     Oxidation/Reduction Potential

Ascorbic acid (AA) is an α-keto-lactone with the chemical formula

$C_6H_6O_8$.[128] It is readily oxidized to dehydroascorbic acid (DHAA) via an intermediate compound, 3-monodehydroascorbate.[126,129,130] This intermediate form has been shown to have free radical properties.[131] Foerster et al demonstrated that mixing AA and DHAA at an acid pH leads to formation of this ascorbic free radical (AFR).[132] Lagercrantz found AFR production in solutions of ascorbate and dissolved oxygen in the pH range of 6.6 to 9.6.[133] Therefore, its ability to remove single electrons from biologically active molecules and transform them into free radicals may allow AA to participate in many enzymatic reactions.[134]

AA can easily be oxidized by cytochrome oxidase plus cytochrome c, and DHAA can be reduced by glutathione. Therefore, some feel that AA may serve to maintain sulfhydryl-containing enzymes reduced by its ability to take part in oxidation/reduction coupling.[129] Intake of large amounts of AA raises the tissue sulfhydryl concentration, suggesting that ascorbate aids in the reduction of disulfides.[135] In support of this theory, Price demonstrated that agents used to reduce sulfhydryls can be replaced with ascorbate.[136] Furthermore, the involvement of the AFR increases the ability of AA to reduce disulfide bonds as compared to simple oxidation/reduction coupling.[137]

C. Ascorbic Acid and Anaphylaxis

Interest in a role for AA in the prevention of asthma developed from previous work suggesting an anti-anaphylactic effect of vitamin C. However, the results of these studies have ranged from the demonstration of marked protection to no effect at all, depending on the system studied and the dosage of vitamin C. Hoffman reported that AA had a protective effect against anaphylaxis in mice.[138] Herxheimer found no protection from

anaphylactic shock in guinea pigs treated with ten to twenty milligrams per kilogram of AA intramuscularly.[139] Dawson and West, however, demonstrated that AA (200 milligrams per kilogram) before antigen challenge protected guinea pigs from anaphylactic shock.[23] Guirgis confirmed this finding and showed that vitamin C (200 milligrams intramuscularly or intraperitonealy) extended the time from inhalation of histamine or antigen aerosol to onset of convulsions in guinea pigs.[140]

D. Ascorbic Acid and Asthma

As early as 1938, Hunt suspected that AA might offer protection to patients with bronchospasm.[22] He reasoned that adrenal medullary insufficiency may somehow be involved in this disease based on the following arguments. Vitamin C is found in significant quantities in the adrenal gland. In fact, Szent Gyorgy used the adrenal as a source when he first isolated AA. Hypersensitivity to proteins can be demonstrated in both adrenalectomized dogs and in asthmatics. Furthermore, eosinophilia is common both in asthmatics and patients with Addison's disease. Finally, adrenaline, well known for its ability to counteract bronchospasm, is produced in the adrenal medulla. However, he found that daily administration of AA (one hundred milligrams orally) for up to eight weeks in asthmatic clinic patients did not improve the amount of their wheezing or the frequency of their attacks. In addition, during asthmatic episodes, 500 to 800 milligrams of vitamin C intramuscularly or intravenously did not decrease symptoms over a period of twenty to thirty minutes.[22]

Stimulated by their earlier work with anaphylactic reactions in guinea pigs, Dawson and West studied AA's protective effect against

bronchospasm in these animals. They demonstrated that 500 milligrams per kilogram intravenously prevented bronchoconstriction induced by intravenous 5-hydroxytryptamine, bradykinin, or histamine. In addition, reducing the dose of AA led to a proportional increase in the amount of bronchospasm. β-adrenergic blockade did not affect AA's protective action.[14]

Zuskin, Lewis, and Bouhuys studied AA's effect both in histamine-induced airway constriction in healthy human subjects and in guinea pig tracheal strips in vitro.[28] Using PEFV curves, they measured the maximal flow rates at fifty and twenty-five percent of vital capacity as parameters of airway obstruction. They demonstrated that five hundred milligrams of AA orally, in contrast to placebo, reduced the amount of bronchospasm provoked by histamine aerosol. Propranolol did not reduce this protection against bronchospasm. Adding AA to an organ bath containing guinea pig tracheal muscle resulted in a dose-related relaxation. AA also reduced histamine-induced contractions in this in vitro system. Both of these effects of vitamin C in guinea pig tracheal muscle in vitro were prevented by β-blockade. They concluded that the differences in response to β-blockade are explained either by a species difference in the mechanism of action of vitamin C in guinea pigs vs man or that the dose of AA in man was sufficient to overcome the β-blockade, in contrast to the guinea pig model. However, when they repeated this experiment using MEF40% (P) to measure bronchospasm in mild asthmatics, no protective effect of AA could be demonstrated.[29] Kordansky et al, in an independent study, showed that vitamin C provided no improvement in ragweed antigen-induced bronchospasm.[30]

Zuskin, Valic, and Bouhuys have provided more evidence for AA's ability to prevent bronchoconstriction by their research with textile dust

exposure. Valic and Zuskin demonstrated that prior administration of vitamin C (five hundred milligrams orally) attenuated the bronchospasm induced by flax dust exposure as measured by FEV_1 and Vmax 50%. This protective effect was also provided by pretreatment with metaproteronol sulfate (alupent[R]) inhaler or diadril, an antihistamine.[142] Zuskin and Bouhuys found that either AA (1 gram) or methdilazine-hydrochloride (8 milligrams), an antihistamine, reduced bronchoconstriction induced by hemp dust extract aerosol inhalation as measured by MEF40% (P). Furthermore, they demonstrated that propranolol potentiated the bronchospastic properties of this textile dust, suggesting that the balance of vagal and β-adrenergic activity is somehow involved.[143]

E. Postulated Mechanisms of Action of AA's Influence on the Contractile State of Smooth Muscle and Its Potential Effect in Asthma

1. Direct Effect on Smooth Muscle

Some researchers theorize a direct effect of vitamin C upon smooth muscle. They provide both direct evidence for an effect of AA as a modulator of smooth muscle tone or indirect evidence, such as the ability of AA to suppress the effect of spasmogens. These arguments have been somewhat confusing as different agents have been shown to have varying effects, depending both on the type of smooth muscle (i.e. ileal, tracheal, etc.) or the species of animal studied. Therefore, one can only speculate from this evidence that AA may have a direct effect upon smooth muscle in the regulation of its contractile state.

a. Early Work

The response of guinea pig ileal muscle to spasmogens has been shown to vary at different times of the year.[25] Dawson et al suggested that variations in the AA content of the diet might account for

these contradictions. Their group has found that the ileal smooth muscle of guinea pigs fed with an AA supplemented diet was ten times more sensitive to acetylcholine and histamine-induced muscle contraction than the muscle of those animals fed a normal diet.[25] In a later experiment, they confirmed that high doses of AA led to inhibition of contraction by these spasmogens, while lower doses actually potentiated their effect.

They have also demonstrated that sodium ascorbate has a direct effect leading to contraction of guinea pig ileal muscle that is probably not related to its ability to alter oxidation/reduction potential since sodium metabisulphite and throurea known reducing agents, were by themselves unable to induce contractions.[27] Furthermore, they confirmed their earlier in vivo experiments demonstrating a protective effect of AA against bronchospasm induced by histamine, bradykinin, 5-hydroxytryptamine, and acetylcholine in guinea pigs. Pretreatment with vitamin C was shown to inhibit bronchial smooth muscle contraction for fifteen minutes, followed by potentiation for fifteen minutes before return to control levels. These effects were unchanged by depleting the animal's tissues of catecholamines or 5-hydroxytryptamine or by β-blockade, suggesting a direct effect upon the tracheal smooth muscle.[27]

      b. Direct Effect of AA on Smooth Muscle - Oxidation/Reduction Potential

In contrast, Ghouri et al suggested that, in some animal models, AA modulates the response of smooth muscle to spasmogens through an alteration of oxidation/reduction potential.[31] Using rabbit and guinea pig ileum, rabbit aortic strips, and rabbit atria, the authors showed that

reducing agents, such as AA and sodium bisulfite, attenuated the normal

contractile effect of acetylcholine, norepinephrine, L-isoproterenol bitar-

trate, and 2-aminoethanol, whereas the oxidizing agent ammonium persulfate

inhibited muscle relaxation by norepinephrine and magnesium. They further

demonstrated that those compounds that produce muscle relaxation, including

AA, were able to reduce the oxidized form of cytochrome c. Those agents

that produced contraction did not show this effect. Therefore, they postu-

lated that the aromatic ring portion of biogenic amines act as reducing agents

and cause muscle relaxation, while the aminoethyl side chains, through their

oxidizing potential, induce contraction. According to this scheme, AA's

capacity to relax certain types of smooth muscle would be a function of its

reducing capacity.

        c.   Direct Effect of AA on Smooth Muscle-Calcium Flux Theory

           Joiner proposed that the direct effect of AA on smooth muscle

might be due to an effect on cellular calcium flux.[26] He demonstrated that

sodium ascorbate stimulated contraction in guinea pig ileal muscle in vitro,

and this effect was partially mimicked by disodium ethylenediamine, a calcium

chelating agent. Sodium ascorbate also potentiated acetylcholine-induced

muscle contractions. Joiner suggested that the direct stimulation by AA may

be due to interactions with calcium in the extracellular space which leads

to decreased levels of calcium near the cell membrane. In response to this

decreased local calcium concentration, calcium located within the cell mem-

brane (and functioning to stabilize it) dissociates and moves extracellularly.

This leads to a change in permeability and an influx of free calcium or

calcium-ascorbate from the extracellular pool. He further speculated than

an ascorbate-calcium interaction in the cell membranes may play a role in the

potentiation of acetylcholine-induced contractions by ascorbate.

Others have demonstrated an effect of AA on calcium flux in

systems other than smooth muscle. Garcia-Sancho et al have demonstrated

that extracellular ascorbate leads to increased potassium flux across the

human red blood cell membrane, an effect similar to that produced by increased

intracellular calcium (the Gardos effect).[144] This change in potassium flux

was also produced by other electron donors, including reduced glutathione.

These authors suggested that ascorbate might alter the oxidation/reduction

state of some membrane component leading to a change in intracellular calcium

which thereby elicits rapid potassium movements. From these various experiments

implicating a calcium-ascorbate interaction in smooth muscle and other systems,

one can only state that a direct effect upon smooth muscle elicited by AA

is potentially mediated by an influence upon calcium flux, possibly by modulating

the oxidation/reduction status of the membrane of the cell or its internal

organelles.

2. AA and Histamine Metabolism

Interested in the protective effect of AA against anaphylaxis,

Dawson and West studied the effect of vitamin C on histamine metabolism.

They demonstrated that a scorbutic state caused increased histamine levels

in guinea pigs as measured by levels in the urine and various tissues. Further-

more, this was shown to be unrelated to a change in histaminase activity.[23]

A series of experiments by Chatterjee et al has helped to

elucidate AA's influence on histamine metabolism. They confirmed that an

AA-free diet in guinea pigs leads to increased levels of histamine in blood, urine, and several other tissues. These increases could be returned to normal levels by a single dose of vitamin C (five milligrams per one hundred grams of body weight). They also demonstrated that there was no change in histamine formation, histaminase activity, or histamine release from mast cells, suggesting a direct effect of AA on histamine degradation.[145] They further reported that histamine levels could be raised in rats and guinea pigs by various stressful conditions, including vaccines, toxoids, cold, heat, pregnancy, drugs, and several diets. AA was able to decrease urinary histamine levels in these animals, indicating in vivo detoxification.[146,147]

The chemical mechanism of this histamine breakdown induced by vitamin C was also addressed by Chatt erjee _et al_. Oxidation of AA to DHAA in the presence of histamine, and copper ion as a catalyst, led to the decomposition of histamine to aspartic acid, ammonia, and carbon dioxide.[32] Monodehydroascorbic acid, the intermediate between AA and DHAA, appeared to be the reactive compound.[32,148] This reaction was demonstrated in biological materials, such as homogenates or slices of different organs of the rat or guinea pig.[32]

3. AA and Cyclic Nucleotides

Ascorbic acid has been shown to have an effect on the levels of the cyclic nucleotides cAMP and cGMP in various experimental models. However, confusion has arisen because the changes in cyclic nucleotide levels induced by vitamin C, and the postulated mechanisms involved, have varied in the different systems studied.

Goldberg _et al_ found that guanylate cyclase activity was stimulated by AA or DHAA in intact guinea pig splenic cells. This activation

appeared to require an oxidant effect. Because DHAA had this action in disrupted cells while AA did not, the authors postulated that AA was converted to DHAA or a free radical intermediate in intact cells and acquired the properties of an oxidizing agent.[149] This theory suggesting intracellular modification of vitamin C was supported by the finding that guanylate cyclase, as opposed to adenylate cyclase, was not predominantly found in the cell membrane, but rather was distributed in soluble and subcellular fractions.[149]

Schoepflin et al studied AA and cyclic nucleotide levels in human platelets. They suggested that AA raises cGMP levels in platelets in a different manner than that by which cholinergic agents raise the level of this cyclic nucleotide in other systems, such as smooth muscle. This was based on the finding that AA's effect, unlike the cholinergic mechanism, was independent of calcium levels.[150,151] They, too, suggest that oxidation might favor an increase in guanylate cyclase acitvity.[151]

Atkinson et al studied the effect of AA on cyclic nucleotide metabolism in human lymphocytes and also found increased levels of cGMP in cells exposed to vitamin C. They found no inhibition of cAMP or cGMP-phosphodiesterase (PDE) activity in this system. They considered several theoretical mechanisms for indirect activation of guanylate cyclase, including auto-oxidation of AA to produce hydrogen peroxide, liberation of fatty acids, prostaglandin production, modulation of redox potential, and free radical formation. However, definitive evidence could not be provided for any of these potential explanations.[35]

Lewin has suggested a scheme for the involvement of vitamin C in cyclic nucleotide metabolism.[152] He has postulated that AA might aid

in the production of epinephrine at three separate stages and therefore
lead to increased activation of adenylate cyclase. These steps include
the involvement of ascorbate as a cofactor in the conversion of dopamine
to norepinephrine, the ability of the reducing power of AA to aid in
methylation of norepinephrine to form epinephrine, and the protection
offered by AA to maintain epinephrine in the active reduced state. In
addition, he has suggested that AA can inhibit PDE's ability to hydrolyze
cAMP by competitively binding to the enzyme. Both cAMP and AA possess ring
structures with negatively charged oxygen molecules that might fit into
the enzyme's active site. This inhibition of PDE has been demonstrated by
other researchers.[153] Tisdale demonstrated that both AA and DHAA are
reversible inhibitors of cAMP - PDE using enzyme obtained from Walker carcinoma
cells in rats.[154] In conclusion, Lewin proposed that vitamin C may increase
cAMP levels by both increasing available epinephrine to stimulate cAMP
production and by inhibiting its enzymatic hydrolysis.

4. AA and Prostaglandins

Nugteren and Hazelhof demonstrated the presence of a compound
(which they designated PGR_2) that is a common intermediate in the formation
of both PGE_2 and $PGF_2\alpha$ in sheep vesicular glands. They also showed that the
isomerization of this intermediate to form PGE_2 required glutathione.[155]
Lands et al confirmed this finding and showed that addition of reduced
glutathione favored production of PGE as opposed to PGF.[156]

Stimulated by evidence that reduced glutathione might lead to
the preferential formation of PGE_2 from cyclic endoperoxide, Puglisi et al
studied the effect of AA on PG metabolism in guinea pig and human uterine
myometrium. In this system, both PGE_2 and $PGF_2\alpha$ stimulate muscle contraction.

The authors found that AA increased smooth muscle contraction, and this was prevented by indomethicin and eicosatetraynoic acid, two inhibitors of PG synthesis. Therefore, they concluded that AA's stimulatory effect was due to PG synthesis. Furthermore, they demonstrated that AA led to a large increase in PGE_2 levels and suggested that vitamin C induced preferential synthesis of PGE_2.[34] Pugh et al observed an inhibitory effect of AA on PGF levels in guinea pig uterine homogenates[33] but did not measure changes in the yield of other PG's.

Puglisi et al studied vitamin C and PG's in guinea pig tracheal smooth muscle. They reported that AA antagonized the airway constriction produced by $PGF_2\alpha$. Furthermore, they demonstrated that AA induced direct smooth muscle relaxation in this system which can be blocked by inhibitors of PG synthesis. This relaxation was found to be proportional to PGE_2 levels measured in the muscle tissue. Muscle samples from animals fed with an AA-free diet contained smaller amounts of PGE_2 and larger quantities of $PGF_2\alpha$ as compared to animals fed a normal diet. Therefore, these authors confirmed the finding that AA leads to enhanced synthesis of PGE_2 as opposed to $PGF_2\alpha$, and they reaffirmed the postulate that vitamin C's effect may be secondary to its ability to maintain glutathione in the reduced state.[157, 158]

VII. DISCUSSION

    A. Discussion of Results

        1. Effect of AA

Our results demonstrate that AA provides protection against airway obstruction in patients with EIB. We found that AA partially reversed the decrement after exercise in all five parameters of pulmonary function and produced a statistically significant attenuation of the post-exertional fall in FVC and FEV_1.

FVC and FEV_1 tend to represent the state of the large airways as opposed to other parameters, such as MEF40% and MEF40% (P), which correlate with small airway status. One explanation for this discrepancy in the response to vitamin C is that the relief of post-exertional airway obstruction offered by AA is predominantly limited to the large airways. Alternatively, the effect of AA may have been more widespread with subtle improvements in smaller airways that were not detected by our parameters because of the large variability of flow rates at low lung volumes.

Previous work has demonstrated contradictory data concerning the ability of vitamin C to reverse histamine-induced airway obstruction as measured by flow rates at low lung volumes using PEFV curves.[28,29] Our results agree with those of Kreisman et al, as no significant change in MEF40% and MEF40% (P) were detected after treatment with AA versus placebo. However, we have demonstrated that AA attenuates the post-exertional decrease in parameters of large airway function in patients with EIB. These parameters representing large airways were not addressed in these previous studies with

histamine challenge. In a separate study by Zuskin, AA was shown to partially prevent decreases in FEV_1 after exposure to flax dust, suggesting that the site of action for AA in this experimental model may be similar to that in EIB.

Kordansky et al found that AA did not protect against ragweed antigen-induced bronchospasm as measured by changes in FEV_1.[30] This may suggest that the mechanism for airway obstruction produced by antigen challenge is different from that produced by exercise, as AA was effective in attenuating decreases in FEV_1, after exercise. It is well known that mast cell degranulation is involved in antigen-induced asthma. If mast cells are also responsible for EIB, AA may be acting at a stage of mast cell function unrelated to its role in antigen-induced asthma.

2. Response to Exercise

Data from the first experimental day reveals that our subjects with EIB demonstrated bronchoconstriction of both large and small airways. However, review of the individual responses of asthmatic subjects shows agreement with the finding of Schachter et al[50] that patients with EIB separate into two distinct groups. Whereas all subjects had large decrements in small airway flow rates (MEF40%, MEF40% (P)), one group had only minimal decreases (< 10%) in parameters measuring large airway function (FVC, FEV_1, PEFR), while a second group demonstrated more severe obstruction in the large airways. We also confirmed the finding of Schachter et al[50] that MEF40% (P) is the most sensitive measure of post-exertional airway obstruction.

In agreement with previous studies, nonasthmatics were shown to have no significant change in pulmonary function after exercise.[50]

This is in contrast to the ability to detect changes in airway dynamics using PEFV curves in healthy subjects with histamine and methacholine challenges.[64]

Both groups revealed improvement in airway dynamics after metaproterenol as compared to baseline measurements ($p < .05$ for FEV_1, PEFR, MEF40%, MEF40% (P) in asthmatics; $p < .05$ for MEF40% and MEF40% (P) in controls). This suggests that metaproterenol is functioning to dilate both large and small airways in the asthmatics, whereas its bronchodilating effect can only be detected in the small airways of healthy subjects.

B. Proposed Models

This demonstration of an antibronchospastic action of vitamin C, an agent able to modulate the effect of several proposed mediators of EIB and with no known influence on the ANS, strongly supports the mediator theory for the etiology of post-exertional asthma. However, the ANS might still play an auxiliary role in regulating the responsiveness of the bronchial smooth muscle to mediators by altering baseline cAMP levels in smooth muscle cells and mast cells. Furthermore, AA's effect may not require a chemical mediator, as vitamin C itself has been shown to directly alter smooth muscle tone.

This protective effect of AA against EIB helps to direct our attention toward those suspected mediators that have been shown to be modulated by vitamin C. Although we have not rigorously eliminated other theories, we have provided sufficient evidence with our review of the literature to narrow the field and postulate certain possible mechanisms. Figure 21 displays four theories to explain the antibronchospastic properties

of AA in patients with EIB.

a. AA as an Antihistamine

Histamine has long been known to stimulate bronchial smooth muscle contraction. There is much evidence to support the theory that the antihistaminic property of vitamin C is central to its role in attenuating EIB. Histamine levels have been demonstrated to be increased in the arterial blood of patients with EIB, and antihistamines have been shown to reduce their symptoms. Chatterjee et al have demonstrated the ability of AA to detoxify histamine both in vitro and in vivo.

b. Direct Effect of AA on Smooth Muscle Tone

A direct effect of vitamin C on smooth muscle cells to relieve bronchospasm can also be postulated. Evidence for this theory is both sparse and indirect. Furthermore, work in this area has been confused by different responses found in different types of smooth muscle. Nevertheless, AA has been shown to both change the response of smooth muscle to spasmogens and to directly alter smooth muscle tone. It has been postulated that these effects are mediated by changes in calcium flux across the smooth muscle cell membrane or by an alteration of the oxidation-reduction potential at the site of action of neurotransmitters. With this indirect, and at times contradictory, evidence, one might speculate that AA alters smooth muscle tone in EIB directly by one or both of these postulated mechanisms.

c. Cyclic Nucleotides

As discussed previously, increases in cAMP levels have been associated with bronchodilatation, suggesting that this cyclic nucleotide might function to prevent bronchospasm. Furthermore, PDE

inhibitors have been shown to relieve airway obstruction in asthmatics. Although no direct effect has been demonstrated, it is postulated that cAMP in some way prevents smooth muscle contraction.

Lewis' theory for vitamin C's ability to modulate cyclic nucleotide levels emphasizes the rise in cAMP levels induced by AA and ignores the confusing data with cGMP and guanylate cyclase. Both by maintaining high levels of epinephrine (which stimulates adenylate cyclase) and by inhibiting PDE, he theorizes that AA might raise cAMP levels in smooth muscle cells and prevent contraction. However, this evidence does not strictly support only the proposition that cyclic nucleotide levels in smooth muscle cells regulate airway tone in EIB. By raising cAMP levels, AA also alters the potential for mast cell degranulation and release of several potential mediators, such as histamine, SRS-A, and kinins. Therefore, vitamin C's ability to maintain increased levels of cAMP in smooth muscle cells may only be an associated finding, while its real effect of decreasing the symptoms of EIB might be due to prevention of mast cell degranulation.

d. PG's

PG's have been shown to modulate airway tone in animals and in man. PGE_2 is a bronchodilator, while $PGF_2\alpha$ stimulates bronchoconstriction.

Because AA leads to the preferential production of PGE_2 as opposed to $PGF_2\alpha$, it has been suggested that regulation of the ratio of these PG's is central to AA's ability to reduce EIB. This action of vitamin C might be mediated by activation of adenylate cyclase or by alteration of calcium flux across smooth muscle cell membranes or the membranes of the

sarcoplasmic reticulum.

C. Speculation and Further Research

This in vivo study has demonstrated the ability of AA to decrease airway obstruction precipitated by exercise. As is usually the case in clinical research, theories of biochemical mechanisms are difficult to rigorously defend based on our results, and one easily feels that he or she is working with a "black box." However, by reviewing the literature of both the in vivo and the in vitro biochemical effects of AA in the prevention of bronchospasm, we have been able to focus attention on the four theories discussed above.

The proposal that AA decreases EIB by degrading histamine seems sound, as histamine has been closely linked to the production of bronchospasm in other forms of asthma. In order to test this hypothesis, measurements of plasma histamine levels might prove useful. One possible experiment could involve exercising patients with EIB after AA or placebo, and attempting to correlate changes in PFT's induced by vitamin C with alterations in plasma histamine levels.

The final common event in all of these theories is contraction of smooth muscle. Because calcium fluxes have been shown to be involved in all forms of muscle contraction, the suggestion that changes in calcium permeability are involved in the attenuation of EIB by vitamin C seems reasonable. This may be a direct action of AA or may be mediated by PG's or cyclic nucleotides. Some researchers have suggested that changes in cAMP and cGMP levels might only be associated with variations in smooth muscle tone and may not actually be causative.[159]

Future biochemical investigation should be directed at the mechanisms of modulation of calcium movements in an attempt to discern the relative involvement of cyclic nucleotides, PG's, or AA itself.

TABLE 1

				Anthropometric Data: Asthmatics			
Subject	Age (years)	Ht (cm)	Wt (kg)	Sex	Allergies	Smoking	Dyspnea* $(0 \to 4)$
1	30	165	77	F	+	-	2-3
2	24	183	63	F	+	-	3
3	33	180	77	M	+	-	1
4	14	165	51	M	+	+	1
5	25	180	61	M	+	-	1-2
6	26	163	87	F	+	-	0
7	21	163	46	F	+	+	3
8	31	166	55	F	+	-	3
9	24	170	61	F	+	-	3
10	27	192	90	M	+	-	1
11	25	165	64	F	+	-	2
12	27	193	91	M	+	-	1
$\bar{X}$	26	173	66				1.8
σ_{n-1}	5	10	15				1.1

* Dyspnea Scale:

0 - 30-40 minutes of strenuous exercise before dyspneic (i.e., jogging)

1 - 10-15 minutes of strenuous exercise before dyspneic (i.e., jogging)

2 - 10-15 minutes of moderate exercise before dyspneic (i.e., volleyball)

3 - 10-15 minutes of minimal exercise before dyspneic (i.e., walking)

4 - dyspneic at rest

TABLE 2   Page 1

PFT's Observed & Expected: Asthmatics

(Baseline Without Drugs)

Subject	FVC (liter)	FVC Expected	% of Expected (liter)	$FEV_{1.0}$ (liter)	$FEV_{1.0}$ Expected	% of Expected (liter/Sec)	PEFR (liter/Sec)
1	3.9	3.9	100.0	3.2	3.1	103.2	7.3
2	5.0	4.8	104.2	2.9	3.9	74.4	5.7
3	4.2	5.4	77.8	1.9	4.2	45.2	4.8
4	3.4	4.0	85.0	2.2	3.3	66.7	4.8
5	5.4	5.6	96.4	3.1	4.4	70.5	6.6
6	3.7	3.9	94.9	2.9	3.1	93.5	6.1
7	3.5	4.0	87.5	3.2	3.3	97.0	6.1
8	2.8	3.9	71.8	1.6	3.1	51.6	3.4
9	3.6	4.2	85.7	2.9	3.4	85.3	6.2
10	5.7	6.2	91.9	4.0	4.8	83.3	9.6
11	3.4	4.0	85.0	2.3	3.2	71.9	5.5
12	3.7	5.7	64.9	2.6	4.5	58.3	5.5
$\bar{X}$			87.1			75.1	
σ_{n-1}			11.5			18.1	

PFT's Observed & Expected: Asthmatics (Cont.)

(Baseline Without Drugs)

Subject	PEFR Expected (liter/Sec)	% of Expected	MEF40% (liter/Sec)	MEF40% (P) (liter/Sec)	Vmax50% (liter/Sec)	Vmax50% Expected (Liter/Sec)	% of Expected
1	6.5	112.3	3.1	2.7	4.1	3.8	109.0
2	7.3	78.1	1.5	1.3	2.0	4.3	46.5
3	9.6	50.0	1.0	0.7	1.3	4.9	26.5
4	7.2	66.7	1.3	1.2	1.7	4.3	39.5
5	9.8	67.3	1.8	1.4	2.2	5.2	42.3
6	6.5	93.8	2.8	1.9	3.5	3.9	89.7
7	6.6	92.4	4.2	4.0	4.9	4.9	122.5
8	6.5	52.3	0.8	0.5	1.1	3.8	28.9
9	6.8	91.2	2.0	2.0	2.9	4.0	72.5
10	10.5	91.4	2.8	2.3	3.7	5.4	68.5
11	6.6	83.3	1.8	1.8	2.1	4.0	52.5
12	10.0	55.3	1.9	2.8	2.4	5.2	46.6
$\bar{X}$		77.8					62.1
$\sigma n-1$		19.6					31.1

TABLE 3

Anthropometric Data: Healthy Subjects

Subject	Age (year)	Ht(cm)	Wt(kg)	Sex	Allergies	Smoking	Dyspnea (0 → 4)
A	24	180	68	M	-	-	0
B	20	173	73	M	-	-	0
C	23	173	58	F	-	-	0
D	25	176	68	M	-	-	0
E	23	188	67	M	+	-	0
F	23	178	74	M	-	-	0
G	22	160	50	F	-	-	0
H	36	170	75	M	-	-	1
I	35	183	74	M	-	-	0
J	21	176	73	M	-	-	0
$\bar{X}$	24	176	68				0.1
$\sigma n-1$	4	8	8				0.3

TABLE 4   Page 4

PFT's Observed & Expected: Healthy Subjects

(Baseline Without Drugs)

Subject	FVC (liter)	FVC Expected	% of Expected (liter)	$FEV_{1.0}$ (liter)	$FEV_{1.0}$ Expected	% of Expected (liter/Sec)	PEFR (liter/Sec)
A	4.5	5.6	80.4	3.5	4.5	77.8	8.9
B	4.0	5.3	75.5	3.3	4.3	76.7	7.3
C	4.0	4.4	90.9	3.9	3.6	108.3	9.1
D	3.7	5.3	69.8	3.4	4.3	79.1	10.5
E	4.6	6.1	75.4	4.4	4.8	91.7	8.4
F	5.2	5.5	94.5	4.9	4.4	111.4	10.0
G	3.7	3.8	97.4	3.2	3.1	103.2	7.9
H	4.9	4.7	104.3	4.5	3.7	121.6	11.4
I	4.8	5.8	82.8	4.5	4.5	100.0	6.2
J	3.9	5.4	72.2	3.2	4.4	72.7	7.1
$\bar{X}$			84.3			94.3	
σ_{n-1}			11.8			17.1	

PFT's Observed & Expected: Healthy Subjects (Cont.)

(Baseline Without Drugs)

Subject	PEFR Expected (liter/Sec)	% of Expected	MEF40% (liter/Sec)	MEF40%(P) (liter/Sec)	Vmax50% (liter/Sec)	Vmax50% Expected (liter/Sec)	% of Expected
A	9.9	89.8	3.9	4.6	4.5	5.2	86.5
B	9.6	76.0	2.8	2.7	3.8	5.2	73.1
C	6.9	131.9	5.1	5.5	6.7	4.2	159.5
D	9.6	109.4	5.4	5.7	7.7	5.1	151.0
E	10.3	81.6	5.7	5.1	6.0	5.4	111.1
F	9.8	102.0	6.4	6.2	7.1	5.2	136.5
G	6.5	121.5	3.6	3.8	4.8	3.9	123.1
H	9.0	126.7	6.1	7.2	7.5	4.5	166.7
I	10.0	62.0	3.8	4.1	4.6	5.2	88.5
J	9.7	73.2	3.0	2.9	3.7	5.2	71.2
$\overline{X}$		97.4					116.7
$\sigma n-1$		24.5					36.0

TABLE 5

Pulmonary Function Response to Exercise Without Drugs: Asthmatics

FVC (liter)

Subject	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post Alupent	% Baseline
1	3.9	100.0	3.9	100.0	3.8	97.4	4.0	102.6
2	5.0	100.0	5.1	102.0	4.4	112.0	5.3	106.0
3	4.8	100.0	5.3	110.4	4.3	89.6	5.7	118.8
4	3.4	100.0	3.0	88.2	2.6	76.5	3.4	100.0
5	5.4	100.0	5.3	98.1	4.7	87.0	5.5	101.9
6	3.7	100.0	3.4	91.9	3.5	94.6	3.7	100.0
7	3.5	100.0	3.5	100.0	3.2	91.4	3.5	100.0
8	2.9	100.0	3.1	110.7	2.6	92.9	3.6	128.6
9	3.6	100.0	3.8	105.5	3.4	94.5	3.8	105.5
10	6.4	100.0	6.1	95.3	6.2	96.9	6.3	98.4
11	3.1	100.0	3.2	103.2	2.6	83.9	3.1	100.0
12	3.7	100.0	3.8	102.7	3.1	83.8	3.3	89.2
$\bar{X}$	4.1	100.0	4.1	100.7	3.7	91.7	4.3	104.3
σ_{n-1}	1.1	0.0	1.0	6.7	1.1	8.9	1.1	10.2
				$t=0.7$		$t=3.1$		$t=1.5$
						$p < .01$		

TABLE 6

Pulmonary Function Response to Exercise Without Drugs: Asthmatics

$FEV_{1.0}$ (liter)

Subject	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post Alupent	% Baseline
1	3.2	100.0	3.2	100.0	2.9	90.6	3.3	103.1
2	2.9	100.0	3.1	106.9	2.5	86.2	3.4	117.2
3	2.4	100.0	2.7	112.5	2.0	83.3	2.9	120.8
4	2.2	100.0	1.9	86.4	1.5	68.2	2.3	104.5
5	3.1	100.0	3.5	112.9	2.5	80.6	3.8	122.6
6	2.9	100.0	2.9	100.0	2.8	96.6	3.1	106.9
7	3.2	100.0	3.3	103.1	3.1	96.9	3.3	103.1
8	1.6	100.0	1.8	112.5	1.4	87.5	2.2	137.5
9	2.9	100.0	2.9	100.0	2.3	79.3	3.2	110.3
10	4.8	100.0	4.7	97.9	4.5	93.7	5.0	104.2
11	1.9	100.0	2.0	105.3	1.4	73.7	1.9	100.0
12	2.6	100.0	2.4	92.3	2.0	76.9	2.1	80.8
$\bar{X}$	2.8	100.0	2.9	102.5	2.4	84.5	3.0	109.3
σ_{n-1}	0.8	0.0	0.8	8.2	0.9	9.1	0.9	14.1
				$t = 1.1$		$t=6.0$ $p< .001$		$t=2.3$ $p<.05$

TABLE 7

Pulmonary Function Response To Exercise Without Drugs: <u>Asthmatic</u>

PEFR (liter/Sec)

Subject	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post Alupent	% Baseline
1	7.3	100.0	7.8	106.8	6.9	94.5	8.2	112.3
2	5.7	100.0	6.0	105.3	5.0	87.7	6.2	108.8
3	5.4	100.0	5.9	109.3	4.2	77.8	5.8	107.4
4	4.5	100.0	4.0	88.9	3.0	66.7	5.6	124.4
5	6.6	100.0	8.1	122.7	5.8	87.9	8.4	127.3
6	6.1	100.0	6.2	101.6	5.8	95.1	6.2	101.6
7	6.1	100.0	6.4	104.9	5.9	96.7	6.6	108.2
8	3.4	100.0	3.8	111.8	3.2	94.1	4.6	135.3
9	6.2	100.0	6.4	103.2	5.3	85.5	6.7	108.1
10	10.6	100.0	10.3	97.2	9.8	92.5	10.4	98.1
11	4.1	100.0	4.4	107.3	2.9	70.7	4.9	119.5
12	5.5	100.0	5.1	92.7	4.2	76.4	4.4	80.0
$\bar{X}$	6.0	100.0	6.2	104.3	5.2	85.5	6.5	110.9
σ_{n-1}	1.8	0.0	1.9	8.9	1.9	10.2	1.8	14.6
				$t=1.7$		$t=5.0$		$t=2.6$
						$p < .001$		$p < .05$

TABLE 8

Pulmonary Function Response to Exercise Without Drugs: Asthmatics

MEF40% (liter/Sec)

Subject	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post Alupent	% Baseline
1	3.1	100.0	3.2	103.1	2.4	77.4	3.8	102.6
2	1.5	100.0	1.7	113.3	1.0	66.7	2.0	133.3
3	1.4	100.0	1.7	121.4	0.7	50.0	1.9	135.7
4	1.4	100.0	1.1	78.6	0.5	35.7	1.6	114.3
5	1.8	100.0	2.6	144.4	1.0	55.6	2.8	155.6
6	2.8	100.0	2.8	100.0	2.3	82.1	3.2	114.3
7	4.2	100.0	4.6	109.5	2.9	69.0	4.6	109.5
8	0.8	100.0	1.2	150.0	0.6	75.0	1.9	237.5
9	2.0	100.0	2.2	110.0	1.4	70.0	3.4	170.0
10	3.6	100.0	3.5	97.2	2.9	80.6	4.1	113.9
11	1.0	100.0	1.2	120.0	0.5	50.0	1.4	140.0
12	1.9	100.0	1.6	84.2	0.8	42.1	1.1	57.9
$\bar{x}$	2.1	100.0	2.3	111.0	1.4	62.9	2.7	133.7
$\sigma n-1$	1.1	0.0	1.1	21.3	0.9	15.7	1.2	42.9
				$t=1.8$		$t=8.3$		$t=2.7$
						$p < .001$		$p<.01$

TABLE 9

Pulmonary Function Response to Exercise Without Drugs: Asthmatics

MEF40% (P) (liter/Sec)

Subject	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post Alupent	% Baseline
1	2.7	100.0	3.0	111.1	1.4	51.9	4.2	155.6
2	1.3	100.0	1.6	123.1	0.8	61.5	2.4	184.6
3	1.4	100.0	1.4	100.0	0.5	35.7	2.1	150.0
4	1.4	100.0	0.9	64.3	0.5	35.7	1.6	114.3
5	1.4	100.0	2.7	192.9	0.7	50.0	3.0	214.3
6	1.9	100.0	2.1	110.5	1.4	73.7	3.2	168.4
7	4.0	100.0	4.5	112.5	2.4	60.0	4.3	107.5
8	0.5	100.0	1.1	220.0	0.4	80.0	2.4	480.0
9	2.0	100.0	2.3	115.0	1.0	50.0	3.4	170.0
10	3.6	100.0	3.3	91.7	2.5	69.4	4.5	125.0
11	0.7	100.0	0.8	114.3	0.3	42.9	1.0	142.9
12	2.8	100.0	1.8	64.3	0.9	32.1	1.4	50.0
$\bar{X}$	2.0	100.0	2.1	118.3	1.1	53.6	2.8	171.9
$\sigma n-1$	1.1	0.0	1.1	45.7	0.7	15.7	1.2	105.7
				$t=1.4$		$P < .001$		$p< .05$
						$t=10.5$		$t=2.4$

TABLE 10

Pulmonary Function Response to Exercise Without Drugs: Healthy Subjects

FVC (liter)

Subject	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post Alupent	% Baseline
1	4.5	100.0	4.5	100.0	4.6	102.2	4.6	102.2
2	4.0	100.0	3.9	97.5	4.1	102.5	4.3	105.0
3	4.0	100.0	3.9	97.5	3.9	97.5	4.0	100.0
4	3.7	100.0	4.0	108.1	3.8	102.7	3.8	102.7
5	4.6	100.0	4.6	100.0	4.4	95.7	4.4	95.7
6	5.2	100.0	5.0	96.2	5.0	96.2	5.3	101.9
7	3.7	100.0	3.6	97.3	3.7	100.0	3.7	100.0
8	4.9	100.0	4.9	100.0	4.9	100.0	5.0	98.0
9	4.8	100.0	4.6	95.8	4.5	93.7	4.6	95.8
10	3.9	100.0	4.4	112.8	4.2	107.7	4.0	102.6
$\bar{X}$	4.3	100.0	4.3	100.5	4.3	99.8	4.4	100.4
σ_{n-1}	0.5	0.0	0.5	5.6	0.4	4.2	0.5	3.1
				$t=0.3$		$t=0.2$		$t=0.4$

TABLE 11

Pulmonary Function Response to Exercise Without Drugs: Healthy Subjects

$FEV_{1.0}$ (liter)

Subject	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post Alupent	% Baseline
1	3.5	100.0	3.6	102.9	3.7	94.7	3.8	108.6
2	3.3	100.0	3.3	100.0	3.4	103.0	3.6	109.1
3	3.9	100.0	3.8	97.4	3.8	97.4	3.8	97.4
4	3.4	100.0	3.7	108.8	3.6	105.9	3.6	105.9
5	4.4	100.0	4.3	97.7	4.3	97.7	4.2	95.5
6	4.9	100.0	4.9	100.0	4.7	95.9	5.2	106.1
7	3.2	100.0	3.2	100.0	3.3	103.1	3.3	103.1
8	4.5	100.0	4.7	104.4	4.7	104.4	4.8	106.7
9	4.5	100.0	4.0	88.9	3.9	86.7	4.1	91.2
10	3.9	100.0	3.8	118.6	3.6	112.5	3.6	112.5
X̄	3.9	100.0	3.9	101.9	3.9	100.1	4.0	103.6
σn-1	0.6	0.0	0.6	7.8	0.5	7.2	0.6	6.8
				t=0.8		t=0.0		t=1.7

TABLE 12

Pulmonary Function Response to Exercise Without Drugs: Healthy Subjects

PEFR (liter/Sec)

Subject	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post Alupent	% Baseline
1	8.9	100.0	9.3	104.5	9.6	107.9	9.7	109.0
2	7.3	100.0	7.5	102.7	7.5	102.7	8.3	113.7
3	9.1	100.0	8.4	92.3	8.5	93.4	8.2	90.1
4	10.5	100.0	10.9	103.8	10.6	100.9	10.0	95.2
5	8.4	100.0	8.8	104.8	8.6	102.4	8.7	103.6
6	10.0	100.0	9.9	99.0	9.7	97.0	9.9	99.0
7	7.9	100.0	8.1	102.5	8.2	103.8	8.1	102.5
8	11.4	100.0	11.6	101.8	11.7	102.6	12.3	107.9
9	6.2	100.0	6.1	98.4	6.0	96.8	6.2	100.0
10	7.1	100.0	9.0	126.8	8.4	118.3	8.5	119.7
X̄	8.7	100.0	9.0	103.7	8.9	102.6	9.0	104.1
σn-1	1.6	0.0	1.6	9.0	1.6	6.9	1.6	8.8
				t=1.3		t=1.2		t=1.5

Pulmonary Function Response to Exercise Without Drugs: Healthy Subjects   TABLE 13

MEF40% (liter/Sec)

Subject	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post Alupent	% Baseline
1	3.9	100.0	3.9	100.0	4.2	107.7	4.7	120.5
2	2.8	100.0	2.8	100.0	3.0	107.1	3.8	135.7
3	5.1	100.0	5.8	113.7	5.6	109.8	6.0	117.6
4	5.4	100.0	7.3	135.2	6.9	127.8	6.2	114.8
5	5.7	100.0	5.9	103.5	5.5	96.5	5.6	98.2
6	6.4	100.0	6.7	104.7	6.7	104.7	6.9	107.8
7	3.6	100.0	3.6	100.0	3.8	105.6	4.1	113.9
8	6.1	100.0	6.6	108.2	6.6	108.2	7.0	114.8
9	3.8	100.0	3.8	100.0	3.7	97.4	4.1	107.9
10	3.0	100.0	4.7	156.7	4.3	143.3	4.5	150.0
$\bar{X}$	4.6	100.0	5.1	112.2	5.0	110.8	5.3	118.1
$\sigma n-1$	1.3	0.0	1.5	19.0	1.4	14.2	1.2	14.8
				t=2.1		t=2.4		t=3.9
						p < .05		p < .01

TABLE 14

Pulmonary Function Response to Exercise Without Drugs: Healthy Subjects

MEF40% (P) (liter/Sec)

Subject	Before Exercise (Baseline)	% Baseline	Immediate Post-Exercise	% Baseline	5 Minute Post-Exercise	% Baseline	Post Alupent	% Baseline
1	4.6	100.0	4.3	93.5	4.6	100.0	5.0	108.7
2	2.7	100.0	2.8	103.7	2.6	96.3	4.1	151.9
3	5.5	100.0	4.7	85.5	4.9	89.1	6.5	118.2
4	5.7	100.0	7.3	128.1	5.5	96.5	7.3	128.1
5	5.1	100.0	4.8	94.1	5.2	102.0	6.3	123.5
6	6.2	100.0	5.8	93.5	6.7	108.1	6.5	104.8
7	3.8	100.0	3.6	94.7	3.7	97.4	4.3	113.2
8	7.2	100.0	7.6	105.6	7.8	108.3	7.9	109.7
9	4.1	100.0	3.5	85.4	3.8	92.7	4.7	114.6
10	2.9	100.0	4.4	178.9	3.9	149.3	3.6	124.1
$\bar{X}$	4.8	100.0	4.9	106.3	4.9	104.0	5.6	119.7
σ_{n-1}	1.4	0.0	1.6	28.4	1.5	17.0	1.5	13.6
				$t=0.7$		$t=0.8$		$t=4.6$
								$p<.01$

TABLE 15

Pulmonary Function Response to Placebo Vs. Drug: FEV, $FEV_{1.0}$, PEFR

Before Exercise

Subject	FVC Placebo (liter)	FVC No Drug (liter)	$FEV_{1.0}$ Placebo (liter)	$FEV_{1.0}$ No Drug (liter)	PEFR Placebo (liter/Sec)	PEFR No Drug (liter/Sec)
1	3.5	3.9	2.8	3.2	6.3	7.3
2	5.0	5.0	2.8	2.9	5.5	5.7
3	4.5	4.8	2.2	2.4	4.9	5.4
4	3.4	3.4	2.4	2.2	5.2	4.5
5	5.1	5.4	2.9	3.1	6.3	6.6
6	3.5	3.7	2.8	2.9	5.9	6.1
7	3.1	3.5	2.9	3.2	6.0	6.1
8	3.5	2.8	2.1	1.6	4.3	3.4
9	3.4	3.6	2.7	2.9	6.1	6.2
10	5.8	6.4	4.2	4.8	9.1	10.6
11	3.2	3.1	2.7	1.9	6.5	4.1
12	3.8	3.7	2.5	2.6	5.7	5.5
$\bar{X}$	4.0	4.1	2.8	2.8	6.0	6.0
σ_{n-1}	0.9	1.1	0.5	0.8	1.2	1.8

TABLE 16

Pulmonary Function Response to Placebo Vs. No Drug: MEF40%, MEF40%(P), Vmax50%

Before Exercise

Subject	MEF40% Placebo (liter/Sec)	MEF40% No Drug (liter/Sec)	MEF40%(P) Placebo (liter/Sec)	MEF40% No Drug (liter/Sec)	Vmax50% Placebo (liter/Sec)	Vmax50% No Drug (liter/Sec)
1	2.5	3.1	2.1	2.7	3.4	4.1
2	1.4	1.5	1.3	1.3	1.9	2.0
3	1.1	1.4	1.0	1.4	1.4	1.8
4	1.6	1.4	1.7	1.4	1.8	1.7
5	1.8	1.8	1.8	1.4	2.2	2.2
6	2.8	2.8	2.6	1.9	3.6	3.5
7	3.7	4.2	4.1	4.0	4.6	4.9
8	1.1	0.8	1.0	0.5	1.5	1.1
9	2.1	2.0	1.9	2.0	2.7	2.9
10	2.8	3.6	2.2	3.6	3.9	4.4
11	2.8	1.0	3.0	0.7	3.6	1.5
12	1.6	1.9	2.0	2.8	2.1	2.4
$\bar{X}$	2.1	2.1	2.1	2.0	2.7	2.7
σ_{n-1}	0.8	1.1	0.9	1.1	1.1	1.2

TABLE 17

Pulmonary Function Response to Placebo Vs. No Drug: FVC, FEV$_{1.0}$, PEFR

Immediate Post-Exercise

Subject	FVC Placebo (liter)	ΔFVC* Placebo (Δ%)	FVC No Drug (liter)	ΔFVC No Drug (Δ%)	FEV$_{1.0}$ Placebo (liter)	ΔFEV$_1$ Placebo (Δ%)	FEV$_{1.0}$ No Drug (liter)	ΔFEV$_{1.0}$ No Drug (Δ%)	PEFR Placebo (liter/sec)	ΔPEFR Placebo (Δ%)	PEFR No Drug (liter/sec)	ΔPEFR No Drug (Δ%)
1	3.7	+5.7	3.9	0.0	2.9	+3.6	3.2	0.0	7.2	+14.3	7.8	+6.8
2	4.8	-4.0	5.1	+2.0	2.8	0.0	3.1	+6.9	5.3	-3.6	6.0	+5.3
3	4.3	-4.4	5.3	+10.4	2.1	-4.5	2.7	+12.5	4.7	-4.1	5.9	+9.3
4	3.1	-8.8	3.0	-11.8	2.1	-12.5	1.9	-13.6	4.2	-19.2	4.0	-11.1
5	5.2	+2.0	5.3	-1.9	3.5	+20.7	3.5	+12.9	7.9	+25.4	8.1	+22.7
6	3.5	0.0	3.4	-8.1	3.0	+7.1	2.9	0.0	6.2	+5.1	6.2	+1.6
7	3.1	0.0	3.5	0.0	3.1	+6.9	3.3	+3.1	6.3	+5.0	6.4	+4.9
8	3.4	+2.9	3.1	+10.7	2.2	+4.8	1.8	+12.5	4.7	+9.3	3.8	+11.8
9	3.5	+2.9	3.8	+5.5	3.0	+11.1	2.9	0.0	6.6	+8.2	6.4	+3.2
10	6.0	+3.4	6.1	-4.7	4.6	+9.5	4.7	-2.1	9.9	+8.8	10.3	-2.8
11	3.2	0.0	3.2	+3.2	2.4	-11.1	2.0	+5.3	5.2	-20.0	4.4	+7.3
12	3.7	-2.6	3.8	+2.7	2.3	-8.0	2.4	-7.7	4.8	-15.8	5.1	-7.3
x̄	4.0	-0.2	4.1	+0.7	2.8	+2.3	2.9	+2.5	6.1	+1.1	6.2	+4.3
σn-1	0.9	4.1	1.0	6.7	0.7	9.9	0.8	8.2	1.6	14.0	1.9	3.9

*Explanation of "ΔPFT" Notation: ΔFVC (Immediately Post-Exercise) = the change in FVC (as a percent of FVC Before Exercise) from Before Exercise to Immediate Post-Exercise

TABLE 18

Pulmonary Function Response to Placebo Vs. No Drug: MEF40%, MEF40%(P)

Immediate Post-Exercise

Subject	MEF40% Placebo (liter/Sec)	ΔMEF40% Placebo (Δ%)	MEF40% No Drug (liter/Sec)	ΔMEF40% No Drug (Δ%)	MEF40%(P) Placebo (liter/Sec)	ΔMEF40%(P) Placebo (Δ%)	MEF40%(P) No Drug (liter/Sec)	ΔMEF40%(P) No Drug (Δ%)
1	2.9	+16.0	3.2	+3.1	2.5	419.0	3.0	+11.1
2	1.4	0.0	1.7	+13.3	1.2	-7.7	1.6	+23.1
3	1.0	-9.1	1.7	+21.4	0.7	-30.0	1.4	0.0
4	1.1	-31.3	1.1	-21.4	1.0	-41.2	1.9	-35.7
5	2.4	+33.3	2.6	+44.4	2.9	+61.1	2.7	+92.9
6	3.3	+17.9	2.8	0.0	3.0	+15.4	2.1	+10.5
7	5.1	+37.8	4.6	+9.5	4.8	+17.1	4.5	+12.5
8	1.3	+18.2	1.2	+50.0	1.3	+30.0	1.1	+120.0
9	2.9	+38.1	2.2	+10.0	2.9	+52.6	2.3	+15.0
10	4.0	+42.9	3.5	-2.8	3.9	+43.6	3.3	-8.3
11	2.2	-21.4	1.2	+20.0	2.5	-16.7	0.8	+14.3
12	1.3	-18.8	1.6	-15.8	1.3	-35.0	1.8	-35.7
$\bar{X}$	2.4	+10.3	2.3	+11.0	2.3	+9.0	2.2	+18.3
$\sigma n-1$	1.3	25.8	1.1	21.3	1.3	34.9	1.0	45.7

TABLE 19

Pulmonary Function Response to Placebo Vs. No Drug: FVC, $FEV_{1.0}$, PEFR

Five-Minutes Post-Exercise

Subject	FVC Placebo (liter)	ΔFVC Placebo (Δ%)	FVC No Drug (liter)	ΔFVC No Drug (Δ%)	$FEV_{1.0}$ Placebo (liter)	$ΔFEV_{1.0}$ Placebo (Δ%)	$FEV_{1.0}$ No Drug (liter)	$ΔFEV_{1.0}$ No Drug (Δ%)	PEFR Placebo (liter/sec)	ΔPEFR Placebo (Δ%)	PEFR No Drug (liter/sec)	ΔPEFR No Drug (Δ%)
1	3.3	-5.7	3.8	-2.6	2.5	-10.7	2.9	-9.4	6.0	-4.8	6.9	-5.5
2	3.9	-22.0	4.4	+12.0	2.1	-25.0	2.5	-13.8	3.9	-29.1	5.0	-12.3
3	3.3	-26.7	4.3	-10.4	1.4	-36.4	2.0	-16.7	3.8	-22.4	4.2	-22.2
4	2.5	-26.5	2.6	-23.5	1.5	-37.5	1.5	-31.8	3.0	-42.3	3.0	-33.3
5	5.2	0.0	4.7	-13.0	2.9	0.0	2.5	-19.4	6.6	+4.8	5.8	-12.1
6	3.6	+2.9	3.5	-5.4	2.8	0.0	2.8	-3.4	5.7	-3.4	5.8	-4.9
7	3.0	-3.2	3.2	-8.6	2.9	0.0	3.1	-3.1	5.7	-5.0	5.9	-3.3
8	3.3	-5.7	2.6	-7.1	2.0	-4.8	1.4	-12.5	4.4	+2.3	3.2	-5.9
9	3.0	-11.8	3.4	-5.5	2.3	-14.8	2.3	-20.7	5.4	-11.5	5.3	-14.5
10	5.7	-1.7	6.2	-3.1	4.3	+2.4	4.5	-6.3	9.1	0.0	9.8	-7.5
11	2.2	-31.3	2.6	-16.1	1.3	-51.9	1.4	-26.3	2.9	-55.4	2.9	-29.3
12	3.1	-18.4	3.1	-16.2	1.7	-32.0	2.0	-23.1	4.0	-29.8	4.2	-23.6
x̄	3.5	-12.5	3.7	-8.3	2.3	-17.6	2.4	-15.5	5.0	-16.4	5.2	-14.5
σn-1	1.0	11.9	1.1	8.9	0.9	18.4	0.9	9.1	1.8	19.3	1.9	10.2

TABLE 20

Pulmonary Function Response to Placebo Vs. No Drug: MEF40%, MEF40% (P)

Five-Minute Post-Exercise

Subject	MEF40% Placebo (liter/Sec)	ΔMEF40% Placebo (Δ%)	MEF40% No Drug (liter/Sec)	ΔMEF40% No Drug (Δ%)	MEF40%(P) Placebo (liter/Sec)	ΔMEF40%(P) Placebo (Δ%)	MEF40%(P) No Drug (liter/Sec)	ΔMEF40%(P) No Drug (Δ%)
1	1.8	-28.0	2.4	-22.6	1.2	-42.9	1.4	-48.1
2	0.5	-64.3	1.0	-33.3	0.3	-76.9	0.8	-38.5
3	0.4	-36.4	0.7	-50.0	0.2	-80.0	0.5	-64.3
4	0.6	-62.5	0.5	-64.3	0.6	-64.7	0.5	-64.3
5	1.6	-11.1	1.0	-44.4	1.4	-22.2	0.7	-50.0
6	2.8	0.0	2.3	-17.9	2.2	-15.4	1.4	-26.3
7	3.5	-5.4	2.9	-31.0	3.5	-14.6	2.4	-40.0
8	1.1	0.0	0.6	-25.0	1.0	0.0	0.4	-20.0
9	1.5	-28.6	1.4	-30.0	1.3	-31.6	1.0	-50.0
10	3.1	+10.7	2.9	-19.4	2.7	+22.7	2.5	-30.6
11	0.2	-92.9	0.5	-50.0	0.2	-93.3	0.3	-57.1
12	0.4	-75.0	0.8	-57.9	0.4	-80.0	0.9	-67.9
X̄	1.5	-32.8	1.5	-37.2	1.3	-41.6	1.1	-46.4
σn-1	1.1	33.8	0.9	15.7	1.1	37.1	0.7	15.7

TABLE 21

Pulmonary Function Response to Placebo Vs. No Drug: FVC, FEV$_{1.0}$, PEFR

Post-Alupent

Subject	FVC Placebo (liter)	ΔFVC Placebo (Δ%)	FVC No Drug (liter)	ΔFVC No Drug (Δ%)	FEV$_{1.0}$ Placebo (liter)	ΔFEV$_{1.0}$ Placebo (Δ%)	FEV$_{1.0}$ No Drug (liter)	ΔFEV$_{1.0}$ No Drug (Δ%)	PEFR Placebo (liter/sec)	ΔPEFR Placebo (Δ%)	PEFR No Drug (liter/sec)	ΔPEFR No Drug (Δ%)
1	3.6	+2.9	4.0	+2.6	3.0	+7.1	3.3	+3.1	7.0	+11.1	8.2	+12.3
2	5.2	+4.0	5.3	+6.0	3.0	+7.1	3.4	+17.2	5.7	+3.6	6.2	+8.8
3	4.8	+6.7	5.7	+18.8	2.2	0.0	2.9	+20.8	4.8	-2.0	5.8	+7.4
4	3.6	+5.9	3.4	0.0	2.7	+12.5	2.3	+4.5	6.7	+28.8	5.6	+24.4
5	5.3	+3.9	5.5	+1.9	3.7	+27.6	3.8	+22.6	8.2	+30.2	8.4	+27.3
6	3.5	0.0	3.7	0.0	3.0	+7.1	3.1	+6.9	5.9	0.0	6.2	+1.6
7	3.2	+3.2	3.5	0.0	3.1	+6.9	3.3	+3.1	6.3	+5.0	6.6	+8.2
8	4.0	+14.3	3.6	+28.6	2.7	+28.6	2.2	+37.5	5.4	+25.6	4.6	+35.3
9	3.6	+5.9	3.8	+5.5	3.2	+18.5	3.2	+10.3	7.0	+14.8	6.7	+8.1
10	5.9	+1.7	6.3	-1.6	4.6	+9.5	5.0	+4.2	10.0	+9.9	10.4	-1.9
11	3.0	-6.3	3.1	0.0	2.4	-11.1	1.9	0.0	5.3	-18.5	4.9	+19.5
12	3.2	-15.8	3.3	-10.8	2.0	-20.1	2.1	-19.2	4.2	-26.3	4.4	-20.1
$\bar{x}$	4.1	+2.2	4.3	+4.3	3.0	+7.8	3.0	+9.3	6.4	+6.9	6.5	+10.9
σ_{n-1}	1.0	7.4	1.1	10.2	0.7	14.0	0.9	14.1	1.6	17.5	1.8	14.7

TABLE 22

Pulmonary Function Response to Placebo Vs. No Drug: MEF40%, MEF40%(P)

Post-Alupent

Subject	MEF40% Placebo (liter/Sec)	ΔMEF40% Placebo (Δ%)	MEF40% No Drug (liter/Sec)	ΔMEF40% No Drug (Δ%)	MEF40%(P) Placebo (liter/Sec)	ΔMEF40%(P) Placebo (Δ%)	MEF40%(P) No Drug (liter/Sec)	ΔMEF40%(P) No Drug (Δ%)
1	3.3	+32.0	3.8	+22.6	3.8	+81.0	4.2	+55.6
2	1.6	+14.3	2.0	+33.3	1.7	+30.8	2.4	+84.6
3	1.1	0.0	1.9	+35.7	1.2	+20.0	2.1	+50.0
4.	2.2	+37.5	1.6	+14.3	2.6	+52.9	1.6	+14.3
5	2.9	+61.1	2.8	+55.6	3.0	+66.6	3.0	+114.3
6	3.2	+14.3	3.2	+14.3	3.7	+42.3	3.2	+168.4
7	4.8	+29.7	4.6	+9.5	4.7	+14.6	4.3	7.5
8	2.1	+90.9	1.9	+137.5	2.6	+160.0	2.4	+380.0
9	3.5	+66.7	3.4	+70.0	4.2	+121.1	3.4	+70.0
10	4.1	+46.4	4.1	+13.9	5.7	+145.5	4.5	+25.0
11	1.8	-35.7	1.4	+40.0	2.4	-20.0	1.0	+42.9
12	0.7	-56.3	1.1	+42.1	0.8	-60.0	1.4	-50.0
$\bar{X}$	2.6	+25.1	2.7	+33.7	3.0	+54.6	2.8	+71.9
σ_{n-1}	1.2	41.8	1.2	42.9	1.5	65.3	1.2	105.7

TABLE 23

*Δ(ΔPFT's) For Placebo Vs. No Drug

Immediate Post-Exercise

Subject	Δ(ΔFVC) Placebo Vs. No Drug (Δ%)	Δ(ΔFEV₁.₀) Placebo Vs. No Drug (Δ%)	Δ(ΔPEFR) Placebo Vs. No Drug (Δ%)	Δ(ΔMEF40%) Placebo Vs. No Drug (Δ%)	Δ(ΔMEF40%(P)) Placebo Vs. No Drug (Δ%)
1	+5.7	+3.6	+7.5	+12.9	+7.9
2	-6.0	-6.9	-8.9	-13.3	-30.8
3	-14.8	-17.1	-13.4	-30.5	-30.0
4	+3.0	+1.1	-8.1	-9.9	-5.5
5	+3.9	+7.8	+2.7	-11.1	-31.8
6	+8.1	+7.1	+3.5	+17.9	+4.9
7	0.0	+3.8	+0.1	+28.3	+4.6
8	-7.8	-7.7	-2.5	-31.8	-90.0
9	-2.6	+11.1	+5.0	+28.1	+37.6
10	+8.1	+11.6	+11.6	+45.7	+51.9
11	-3.2	-16.4	-27.3	-41.4	-31.0
12	-5.3	-0.3	-8.5	-3.0	+0.7
$\bar{X}$	-0.9	-0.2	-3.2	-0.7	-9.3
$\sigma n-1$	7.0	9.8	10.7	27.3	37.1
	t=0.5	t=0.1	t=1.0	t=0.1	t=0.9

*Explanation of Δ(ΔPFT) Notation: Δ(ΔFVC) for Placebo Vs. No Drug = ΔFVC Placebo - ΔFVC No Drug

TABLE 24

Δ(ΔPFT's) For Placebo Vs. No Drug

Five-Minutes Post-Exercise

Subject	Δ(ΔFVC) Placebo Vs. No Drug (Δ%)	Δ(ΔFEV1.0) Placebo Vs. No Drug (Δ%)	Δ(ΔPEFR) Placebo Vs. No Drug (Δ%)	Δ(ΔMEF40%) Placebo Vs. No Drug (Δ%)	Δ(ΔMEF40%(P)) Placebo Vs. No Drug (Δ%)
1	-3.1	-1.3	+0.7	-5.4	+5.2
2	-34.0	-11.2	-16.8	-31.0	-38.4
3	-16.3	-19.7	-0.2	+13.6	-15.0
4	-3.0	-5.7	-9.0	+1.8	-0.4
5	+13.0	-19.4	16.9	+33.3	+27.8
6	+8.3	+3.4	+1.5	+17.9	+10.9
7	+5.4	+3.1	-1.7	+25.6	+25.4
8	+1.4	+7.7	+8.2	+25.0	+20.0
9	-6.3	+5.9	+3.0	+1.4	+18.4
10	+1.4	+8.7	+7.5	+30.1	+53.3
11	-15.2	-25.6	-26.1	-42.9	-36.2
12	-2.2	-8.4	-6.2	-17.1	-12.1
X̄	-4.2	-2.0	-1.9	+4.4	+4.9
σn-1	12.7	12.8	11.5	24.7	27.0
	t=1.2	t=0.5	t=0.6	t=0.6	t=0.6

TABLE 25

Δ(ΔPFT's) For Placebo Vs. No Drug

Post-Alupent

Subject	Δ(ΔFVC) Placebo Vs. No Drug (Δ%)	Δ(ΔFEV1.0) Placebo Vs. No Drug (Δ%)	Δ(ΔPEFR) Placebo Vs. No Drug (Δ%)	Δ(ΔMEF40%) Placebo Vs. No Drug (Δ%)	Δ(ΔMEF40%(P)) Placebo Vs. No Drug (Δ%)
1	+0.3	+4.0	-1.2	+9.4	+25.4
2	-2.0	-10.1	-5.2	-19.0	-53.8
3	-12.1	-20.8	-9.4	-35.7	-30.0
4	+5.9	+8.0	+4.9	+23.2	+38.6
5	+2.0	+5.0	+2.9	+5.5	-47.7
6	0.0	+0.2	-1.6	0.0	-26.1
7	+3.2	+3.8	+3.2	+20.2	+7.1
8	-14.3	-8.9	-9.7	-46.6	-220.0
9	+0.4	+8.2	+6.7	-3.2	+51.1
10	+3.3	+5.3	+11.8	+32.5	+120.5
11	-6.3	-11.1	-38.0	-75.7	-62.9
12	-5.0	-0.9	-6.2	-14.2	-10.0
x̄	-2.1	-1.4	-4.1	-8.6	-17.3
σn-1	6.2	9.2	12.5	31.5	82.5
	t=1.2	t=0.5	t=1.1	t=1.0	t=0.7

TABLE 26

Pulmonary Function Response to Vitamin C Vs. Placebo: FVC, FEV1.0, PEFR

Subject	FVC Vitamin C (liter)	FVC Placebo (liter)	Before Exercise FEV$_{1.0}$ Vitamin C (liter)	FEV$_{1.0}$ Placibo (liter)	PEFR Vitamin C (liter/sec)	PEFR Placebo (liter/sec)
1	3.7	3.5	2.8	2.8	6.1	6.3
2	5.1	5.0	3.0	2.8	5.7	5.5
3	4.3	4.5	2.0	2.2	4.3	4.9
4	3.4	3.4	2.1	2.4	4.1	5.2
5	4.5	5.1	2.4	2.9	6.3	6.3
6	3.5	3.5	2.7	2.8	5.2	5.9
7	2.7	3.1	2.3	2.9	5.3	6.0
8	3.1	3.5	1.8	2.1	3.9	4.3
9	3.2	3.4	2.5	2.7	5.4	6.1
10	6.1	5.8	4.4	4.2	9.7	9.1
11	2.9	3.2	2.1	2.7	5.1	6.5
12	3.7	3.8	2.5	2.5	5.6	5.7
$\bar{x}$	3.9	4.0	2.6	2.8	5.6	6.0
σn1	1.0	0.9	0.7	0.5	1.5	1.2

TABLE 27

Pulmonary Function Response to Vitamin C Vs. Placebo: MEF40%, MEF40%(P), Vmax50%

Before Exercise

Subject	MEF40% Vitamin C (liter/Sec)	MEF40% Placebo (liter/Sec)	MEF40%(P) Vitamin C (liter/Sec)	MEF40%(P) Placebo (liter/Sec)	Vmax50% Placebo (liter/Sec)	Vmax50% Vitamin C (liter/Sec)
1	2.2	2.5	1.7	2.1	3.0	3.4
2	1.4	1.4	1.2	1.3	1.9	1.9
3	0.9	1.1	0.8	1.0	1.2	1.4
4	1.2	1.6	1.2	1.7	1.6	1.8
5	1.3	1.8	1.1	1.8	1.7	2.2
6	2.3	2.8	1.5	2.6	3.1	3.6
7	2.3	3.7	2.4	4.1	2.9	4.6
8	1.0	1.1	0.9	1.0	1.3	1.5
9	1.8	2.1	1.8	1.9	2.1	2.7
10	2.9	2.8	3.0	2.2	3.9	3.9
11	1.6	2.8	1.8	3.0	2.2	3.6
12	1.8	1.6	2.4	2.0	2.3	2.1
$\bar{X}$	1.7	2.1	1.7	2.1	2.3	2.7
σ_{n-1}	0.6	0.8	0.7	0.9	0.8	1.1

Pulmonary Function Response to Vitamin C Vs. Placebo: FVC, FEV1.0, PEFR   Table 28

Immediate Post-Exercise

Subject	FVC Vitamin C (liter)	ΔFVC Vitamin C (Δ%)	FVC Placebo (liter)	ΔFVC Placebo (Δ%)	FEV$_{1.0}$ Vitamin C (liter)	ΔFEV$_{1.0}$ Vitamin C (Δ%)	FEV$_{1.0}$ Placebo (liter)	ΔFEV$_{1.0}$ Placebo (Δ%)	PEFR Vitamin C (liter/sec)	ΔPEFR Vitamin C (Δ%)	PEFR Placebo (liter/sec)	ΔPEFR Placebo (Δ%)
1	3.5	-5.4	3.7	+5.7	2.7	-3.6	2.9	+3.6	6.5	+6.6	7.2	+14.3
2	4.8	-5.9	4.8	-4.0	3.2	-6.7	2.8	0.0	5.7	0.0	5.3	-3.6
3	4.7	+9.3	4.3	-4.4	2.2	+10.0	2.1	-4.5	5.0	+16.3	4.7	-4.1
4	3.3	-3.0	3.1	-8.8	2.3	+9.5	2.1	-12.5	4.5	+9.8	4.2	-19.2
5	4.7	+4.4	5.2	+2.0	2.8	+16.7	3.5	+20.7	7.7	+22.2	7.9	+25.4
6	3.3	-5.7	3.5	0.0	2.7	0.0	3.0	+7.1	5.4	+3.8	6.2	+5.1
7	2.8	+3.7	3.1	0.0	2.6	+13.0	3.1	+6.9	5.6	+5.7	6.3	+5.0
8	3.4	+9.7	3.4	+2.9	2.2	+22.2	2.2	+4.8	4.8	+23.1	4.7	+9.3
9	3.5	+9.4	3.5	+2.9	2.9	+16.0	3.0	+11.1	6.4	+18.5	6.6	+8.2
10	6.2	+1.6	6.0	+3.4	4.6	+4.5	4.6	+9.5	10.0	+3.1	9.9	+8.8
11	3.3	+13.8	3.2	0.0	2.5	+19.0	2.4	-11.1	6.0	+17.6	5.2	-20.0
12	3.9	+5.4	3.7	-2.6	2.4	-4.0	2.3	-8.0	5.2	-7.1	4.8	-15.8
x̄	4.0	+3.1	4.0	-0.2	2.8	+8.1	2.8	+2.3	6.1	+10.0	6.1	+1.1
σn1	1.0	6.8	0.9	4.1	0.7	9.8	0.7	9.9	1.5	9.5	1.6	14.0

TABLE 29

Pulmonary Function Response to Vitamin C Vs. Placebo: MEF40%, MEF40%,(P)

Immediate Post-Exercise

Subject	MEF40% Vitamin C (liter/sec)	ΔMEF40% Vitamin C (Δ%)	MEF40% Placebo (liter/sec)	ΔMEF40% Placebo (Δ%)	MEF40%(P) Vitamin C (liter/sec)	ΔMEF40%(P) Vitamin C (Δ%)	MEF40%(P) Placebo (liter/sec)	ΔMEF40%(P) Placebo (Δ%)
1	2.1	-4.5	2.9	+16.0	1.7	0.0	2.5	+19.0
2	1.3	-7.1	1.4	0.0	1.3	+8.3	1.2	-7.7
3	1.2	+33.3	1.0	-9.1	0.8	0.0	0.7	-30.0
4	1.5	+25.0	1.1	-31.3	1.4	+16.7	1.0	-41.2
5	2.0	+53.8	2.4	+33.3	1.9	+72.7	1.4	+61.1
6	2.3	0.0	3.3	+17.9	2.1	+40.0	3.0	+15.4
7	3.5	+52.2	5.1	+37.8	4.1	+70.8	4.8	+17.1
8	1.5	+50.0	1.3	+18.2	1.7	+88.9	1.3	+30.0
9	2.6	+44.4	2.9	+38.1	3.1	+72.2	2.9	+52.6
10	3.2	+10.3	4.0	+42.9	3.4	+13.3	3.9	+43.6
11	2.9	+81.3	2.2	-21.4	3.4	+88.9	2.5	-16.7
12	1.6	-11.1	1.3	-18.8	1.7	-29.2	1.3	-35.0
$\bar{x}$	2.2	+27.3	2.4	+10.3	2.2	+36.9	2.2	+9.0
$\sigma n-1$	0.9	29.8	1.3	25.8	1.0	40.4	1.3	34.9

TABLE 30

Pulmonary Function Response to Vitamin C Vs. Placebo: FVC, $FEV_{1.0}$, PEFR

Five-Minute Post-Exercise

Subject	FVC Vitamin C (liter)	ΔFVC Vitamin C (Δ%)	FVC Placebo (liter)	ΔFVC Placebo (Δ%)	$FEV_{1.0}$ Vitamin C (liter)	$ΔFEV_{1.0}$ Vitamin C (Δ%)	$FEV_{1.0}$ Placebo (liter)	$ΔFEV_{1.0}$ Placebo (Δ%)	PEFR Vitamin C (liter/sec)	ΔPEFR Vitamin C (Δ%)	PEFR Placebo (liter/sec)	ΔPEFR Placebo (Δ%)
1	3.7	0.0	3.3	-5.7	2.6	-7.1	2.5	-10.7	5.8	-4.9	6.0	-4.8
2	4.4	-13.7	3.9	-22.0	2.6	-13.3	2.1	-25.0	4.8	-15.8	3.9	-29.1
3	3.8	-11.6	3.3	-26.7	1.6	-20.0	1.4	-36.4	3.7	-14.0	3.8	-22.4
4	3.3	-3.0	2.5	-26.5	2.0	-4.8	1.5	-37.5	4.3	+4.9	3.0	-42.3
5	4.8	+6.7	5.2	0.0	2.4	0.0	2.9	0.0	5.5	-19.0	6.6	+4.8
6	3.4	-2.9	3.6	+2.9	2.4	-11.1	2.8	0.0	5.0	-3.8	5.7	-3.4
7	2.5	-7.4	3.0	-3.2	2.2	-4.3	2.9	0.0	5.0	-5.7	5.7	-5.0
8	2.9	-6.5	3.3	-5.7	1.8	0.0	2.0	-4.8	3.9	0.0	4.4	+2.3
9	3.1	-3.1	3.0	-11.8	2.3	-8.0	2.3	-14.8	5.4	0.0	5.4	-11.5
10	5.9	-3.3	5.7	-1.7	4.4	0.0	4.3	+2.4	9.5	-2.1	9.1	0.0
11	2.3	-20.7	2.1	-31.3	1.4	-33.3	1.3	-51.9	2.9	-41.2	2.9	-55.4
12	3.3	-10.8	3.1	-18.4	2.0	-20.0	1.7	-32.0	4.1	-26.8	4.0	-29.8
$\bar{x}$	3.6	-6.4	3.5	-12.5	2.3	-10.2	2.3	-17.6	5.0	-10.7	5.0	-16.4
$σn-1$	1.0	7.1	1.0	11.9	0.8	10.1	0.9	18.4	1.7	13.3	1.8	19.3

Pulmonary Function Response to Vitamin C Vs. Placebo: MEF40%, MEF40%(P)   TABLE 31

Five-Minute Post-Exercise

Subject	MEF40% Vitamin C (liter/sec)	ΔMEF40% Vitamin C (Δ%)	MEF40% Placebo (liter/sec)	ΔMEF40% Placebo (Δ%)	MEF40%(P) Vitamin C (liter/sec)	ΔMEF40%(P) Vitamin C (Δ%)	MEF40%(P) Placebo (liter/sec)	ΔMEF40%(P) Placebo (Δ%)
1	1.7	-22.7	1.8	-28.0	0.9	-47.9	1.2	-42.9
2	1.0	-28.6	0.5	-64.3	0.7	-41.7	0.3	-76.9
3	0.6	-33.3	0.4	-36.4	0.4	-50.0	0.2	-80.0
4	1.2	0.0	0.6	-62.5	1.2	0.0	0.6	-64.7
5	1.3	0.0	1.6	-11.1	0.7	-36.4	1.4	-22.2
6	1.5	-34.8	2.8	0.0	0.8	-46.7	2.2	-15.4
7	1.7	-26.1	3.5	-5.4	2.2	-8.3	3.5	-14.6
8	0.9	-10.0	1.1	0.0	0.8	-11.1	1.0	0.0
9	1.6	-11.1	1.5	-28.6	1.4	-22.2	1.3	-31.6
10	2.6	-10.3	3.1	+10.7	2.5	-16.7	2.7	+22.7
11	0.5	-68.8	0.2	-92.9	0.6	-66.7	0.2	-93.3
12	0.9	-50.0	0.4	-75.0	0.8	-66.7	0.4	-80.0
x̄	1.3	-24.6	1.5	-32.8	1.1	-34.5	1.3	-41.6
σn-1	0.6	20.5	1.1	33.8	0.6	22.5	1.1	37.1

TABLE 32

Pulmonary Function Response to Vitamin C Vs. Placebo: FVC, $FEV_{1.0}$, PEFR

Post-Alupent

Subject	FVC Vitamin C (liter)	ΔFVC Vitamin C (Δ%)	FVC Placebo (liter)	ΔFVC Placebo (Δ%)	$FEV_{1.0}$ Vitamin C (liter)	$ΔFEV_{1.0}$ Vitamin C (Δ%)	$FEV_{1.0}$ Placebo (liter)	$ΔFEV_{1.0}$ Placebo (Δ%)	PEFR Vitamin C (liter/sec)	ΔPEFR Vitamin C (Δ%)	PEFR Placebo (liter/sec)	ΔPEFR Placebo (Δ%)
1	3.8	+2.7	3.6	+2.9	3.1	+10.7	3.0	+7.1	7.2	+18.0	7.0	+11.1
2	5.3	+3.9	5.2	+4.0	3.3	+10.0	3.0	+7.1	6.1	+7.0	5.7	+3.6
3	5.0	+16.3	4.8	+6.7	2.3	+65.0	2.2	0.0	5.0	+16.3	4.8	-2.0
4	3.3	-3.0	3.6	+5.9	2.5	+19.0	2.7	+12.5	6.2	+51.2	6.7	+28.8
5	5.6	+24.4	5.3	+3.9	3.9	+62.5	3.7	+27.6	8.0	+27.0	8.2	+30.2
6	3.5	0.0	3.5	0.0	2.8	+3.7	3.0	+7.1	5.6	+7.7	5.9	0.0
7	2.8	+3.7	3.2	+3.2	2.7	+17.4	3.1	+6.9	5.4	+1.9	6.3	+5.0
8	3.8	+22.6	4.0	+14.3	2.6	+44.4	2.7	+28.6	5.3	+35.9	5.4	+25.6
9	3.6	+12.5	3.6	+5.9	3.1	+24.0	3.2	+18.5	6.7	+24.1	7.0	+14.8
10	6.4	+4.9	5.9	+1.7	4.9	+11.4	4.6	+9.5	11.0	+13.4	10.0	+9.9
11	3.0	+3.4	3.0	-6.3	2.3	+9.5	2.4	-11.1	5.9	+15.7	5.3	-18.5
12	3.6	-2.7	3.2	-18.8	2.2	-12.0	2.0	-20.0	4.3	-23.0	4.2	-26.3
x̄	4.1	+7.4	4.1	+2.2	3.0	+22.1	3.0	+7.8	6.4	+16.3	6.4	+6.9
σn-1	1.1	9.3	1.0	7.4	0.8	23.5	0.7	14.0	1.8	18.3	1.6	17.5

TABLE 33

Pulmonary Function Response to Vitamin C Vs. Placebo:MEF40%, MEF40%(P)

Post-Alupent

Subject	MEF40% Vitamin C (liter/sec)	ΔMEF40% Vitamin C (Δ%)	MEF40% Placebo (liter/sec)	ΔMEF40% Placebo (Δ%)	MEF40%(P) Vitamin C (liter/sec)	ΔMEF40%(P) Vitamin C (Δ%)	MEF40%(P) Placebo (liter/sec)	ΔMEF40%(P) Placebo (Δ%)
1	3.1	+40.9	3.3	+32.0	2.8	+52.9	3.8	+81.0
2	2.0	+42.9	1.6	+14.3	2.2	+83.3	1.7	+30.8
3	1.3	+44.0	1.1	0.0	1.5	+87.5	1.2	+20.0
4	1.9	+58.3	2.2	+37.5	2.4	+100.0	2.6	+52.9
5	3.6	+176.9	2.9	+61.1	3.8	+245.5	3.0	+66.6
6	2.9	+26.1	3.2	+14.3	2.3	+53.3	3.7	+42.3
7	4.6	+100.0	4.8	+29.7	4.6	+91.7	4.7	+14.6
8	2.1	+110.0	2.1	+90.9	2.7	+200.0	2.6	+160.0
9	3.3	+83.3	3.5	+66.7	4.4	+144.4	4.2	+121.1
10	3.7	+27.6	4.1	+46.4	4.7	+56.7	5.7	+145.5
11	1.9	+18.8	1.8	-35.7	2.4	+33.3	2.4	-20.0
12	1.2	-33.3	0.7	-56.3	1.0	-58.3	0.8	-6.0
x̄	2.6	+58.0	2.6	+25.1	2.9	+90.9	3.0	+54.6
σn-1	1.1	53.7	1.2	41.8	1.2	78.9	1.5	65.3

TABLE 34

Δ(ΔPFT's) For Vitamin C Vs. Placebo

Immedidate Post-Exercise

Subject	Δ(ΔFVC) Vitamin C Vs. Placebo (Δ%)	Δ(ΔFEV1.0) Vitamin C Vs. Placebo (Δ%)	Δ(ΔPEFR) Vitamin C Vs. Placebo (Δ%)	Δ(ΔMEF40%) Vitamin C Vs. Placebo (Δ%)	Δ(ΔMEF40%(P)) Vitamin C Vs. Placebo (Δ%)
1	-11.1	-7.2	+7.7	-20.5	-19.0
2	-1.9	-6.7	+3.6	-7.1	+16.0
3	+13.7	+14.5	+20.4	+42.4	+30.0
4	+5.8	+22.0	+29.0	+56.3	+57.9
5	+2.4	-4.0	-3.2	+20.5	+11.6
6	-5.7	-7.1	-1.3	-17.9	+24.6
7	+3.7	+6.1	+0.7	+14.4	+53.7
8	+6.8	+17.4	+13.3	+31.8	+58.9
9	+6.5	+4.9	+10.3	+6.3	+19.6
10	-1.8	-5.0	-5.7	-32.1	-30.3
11	+13.8	+30.1	+37.6	+102.7	+105.6
12	+8.0	+4.0	+8.7	+7.7	+5.8
X̄	+3.4	+5.8	+8.8	+17.0	+27.9
σn-1	7.5	12.7	14.1	37.3	37.1
	t=1.6	t=1.5	t=2.2	t=1.6	t=2.6
			p<.05		p<.05

TABLE 35

Δ(ΔPFT's) For Vitamin C vs. Placebo

Five-Minute Post-Exercise

Subject	Δ(ΔFVC) Vitamin C Vs. Placebo (Δ%)	Δ(ΔFEV$_{1.0}$) Vitamin C Vs. Placebo (Δ%)	Δ(ΔPEFR) Vitamin C Vs. Placebo (Δ%)	Δ(ΔMEF40%) Vitamin C Vs. Placebo (Δ%)	Δ(ΔMEF40%(P)) Vitamin C Vs. Placebo (Δ%)
1	+5.7	+3.6	-0.1	+5.3	-5.0
2	+8.3	+11.7	+13.3	+35.7	+35.2
3	+15.1	+16.4	+8.4	+3.1	+30.0
4	+23.5	+32.7	+47.2	+62.5	+64.7
5	+6.7	0.0	-23.8	+11.1	-14.2
6	-5.8	-11.1	-0.4	-34.8	-31.3
7	-4.2	-4.3	-0.7	-20.7	+6.3
8	-0.8	+4.8	-2.3	-10.0	-11.1
9	+8.7	+6.8	-1.5	+17.5	+9.4
10	-1.6	-2.4	-2.1	-21.0	-39.4
11	+10.6	+18.6	+14.2	+24.1	+26.6
12	+7.6	+12.0	+3.0	+25.0	+13.3
$\bar{x}$	+6.1	+7.4	+5.7	+8.2	+7.0
$\sigma n-1$	8.4	11.8	16.5	27.4	29.6
	t=2.5	t=2.2	t=1.2	t=1.0	t=0.8
	p<.05	p<.05			

TABLE 36

Δ(ΔPFT's) For Vitamin C Vs. Placebo

Post-Alupent

Subject	Δ(ΔFVC) Vitamin C Vs. Placebo (Δ%)	Δ(ΔFEV₁.₀) Vitamin C Vs. Placebo (Δ%)	Δ(ΔPEFR) Vitamin C Vs. Placebo (Δ%)	Δ(ΔMEF40%) Vitamin C Vs. Placebo (Δ%)	Δ(ΔMEF40%(P)) Vitamin C Vs. Placebo (Δ%)
1	-0.2	+3.6	+6.9	+8.9	-28.1
2	-0.1	+2.9	+3.4	+28.6	+52.5
3	+9.6	+65.0	+18.3	+44.4	+67.5
4	-8.9	+6.5	+22.4	+20.8	+47.1
5	+20.5	+34.9	-3.2	+115.8	+178.9
6	0.0	-3.4	+7.7	+11.8	+11.0
7	+0.5	+10.5	-3.1	+70.3	+77.1
8	+8.3	+15.8	+10.3	+19.1	+40.0
9	+6.6	+5.5	+9.3	+16.6	+23.3
10	+3.2	+1.9	+3.5	-18.8	-88.8
11	+9.9	+20.6	+34.2	+54.5	+53.3
12	+13.1	+8.0	+3.3	+23.0	+1.7
x̄	+5.2	+14.3	+9.4	+32.9	+36.3
σn1	7.7	18.9	10.9	34.7	64.3
	t=2.4	t=2.6	t=3.0	t=3.3	t=2.0
	p<.05	p<.05	p<.02	p<.01	

TABLE 37

PAIRED t-TESTS FOR $\Delta(\Delta$ PFT'S)

Subject	Placebo vs. No Drug			Vitamin C vs. Placebo		
	t Immediate Post Exercise	t Five-Minute Post-Exercise	t Post-Alupent	t Immediate Post-Exercise	t Five-Minute Post-Exercise	t Post-Alupent
$\Delta(\Delta$ FVC)	0.5	1.2	1.2	1.6	2.5 $p < .05$	2.4 $p < .05$
$\Delta(\Delta$ FEV$_{1.0}$)	0.1	0.5	0.5	1.5	2.2 $p < .05$	2.6 $p < .05$
$\Delta(\Delta$ PEFR)	1.0	0.6	1.1	2.2 $p < .05$	1.2	3.0 $p < .05$
$\Delta(\Delta$ MEF$_{40\%}$)	0.1	0.6	1.0	1.6	1.0	3.3 $p < .02$
$\Delta(\Delta$ MEF$_{40\%}$(P))	0.9	0.6	0.7	2.6 $p < .05$	0.8	2.0

Analysis of Variance of PFT's on Three Experimental Days

For Asthmatic Subjects

PFT's	No Drug	Placebo	Vitamin C	F^*
Mean Baseline FVC (liter)	4.1	4.0	3.8	0.2
Mean Baseline $FEV_{1.0}$ (liter)	2.8	2.8	2.6	0.2
Mean Baseline PEFR (liter/Sec)	6.0	6.0	5.6	0.3
Mean Baseline MEF40% (liter/Sec)	2.1	2.1	1.7	0.9
Mean Baseline MEF40% (P) (liter/Sec)	2.0	2.1	1.7	0.6
Mean Baseline Vmax50% (liter/Sec)	2.7	2.7	2.3	0.1

Critical F value for $p < .05 = 3.29$

TABLE 38

FIGURE 1

SCHEMATIC REPRESENTATION OF DAILY ROUTINE

| PFT'S | FIVE-SEVEN MINUTES
EXERCISE | PFT'S | FIVE MINUTES | PFT'S | ALUPENT | TEN MINUTES | PFT'S |
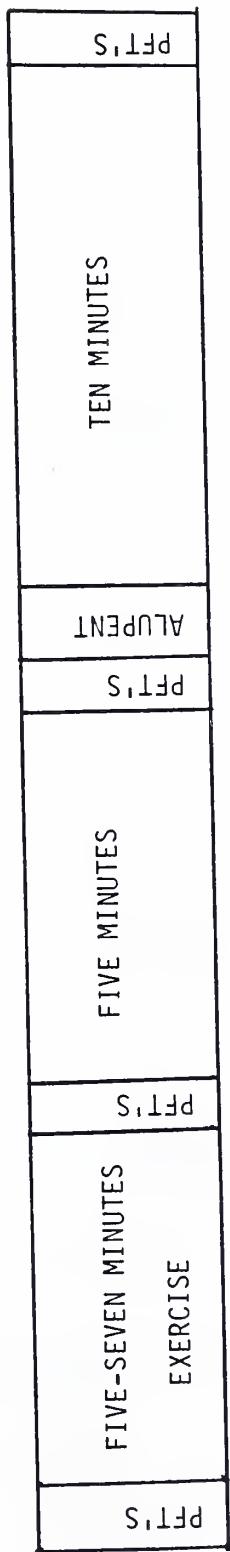
MEFV   REPRESENTATIVE PAIR OF MEFV & PEFV CURVES   FIGURE 2

SUBJECT 9, WITHOUT DRUGS, POST-ALUPENT
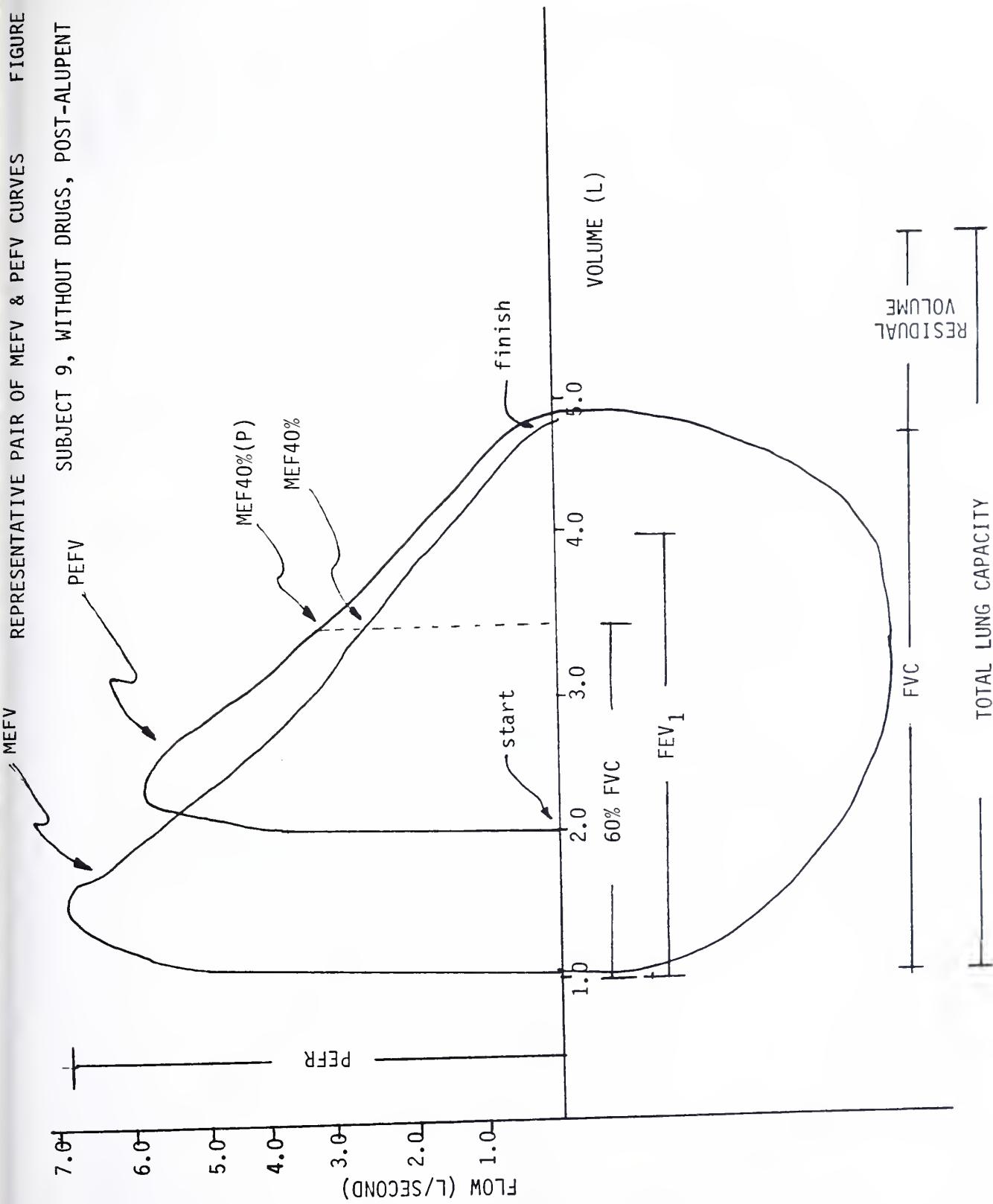
REPRESENTATIVE MEFV & PEFV CURVES: SUBJECT 9, WITHOUT DRUGS
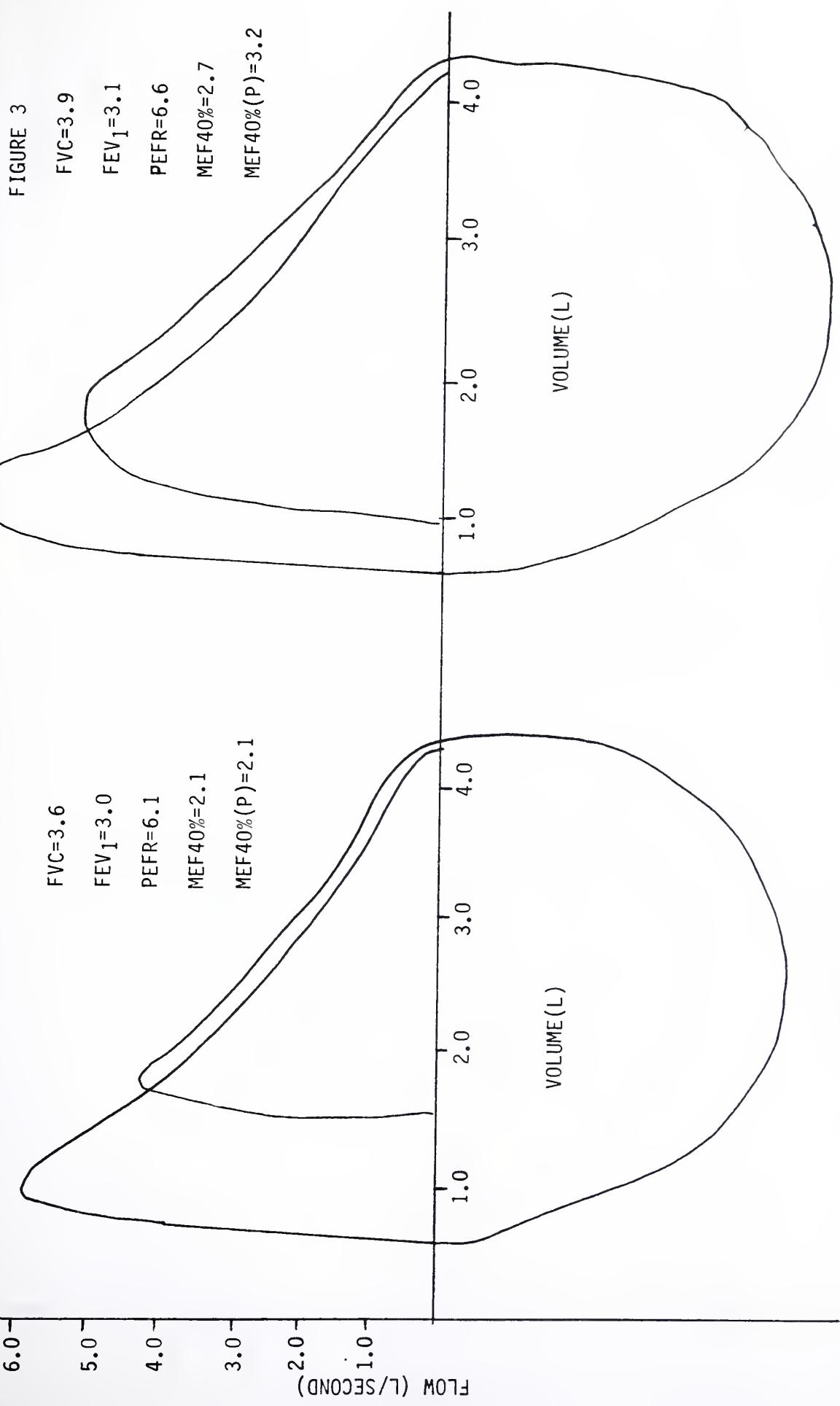
FIGURE 3

FVC=3.9
FEV_1=3.1
PEFR=6.6
MEF40%=2.7
MEF40%(P)=3.2

FVC=3.6
FEV_1=3.0
PEFR=6.1
MEF40%=2.1
MEF40%(P)=2.1

IMMEDIATE POST-EXERCISE

BASELINE

VOLUME(L)

VOLUME(L)

FLOW (L/SECOND)

REPRESENTATIVE MEFV & PEFV CURVES: SUBJECT 9, WITHOUT DRUGS

FIGURE 4

FVC=3.8
FEV$_1$=3.3
PEFR=6.8
MEF40%=3.1
MEF40%(P)=3.9

FVC=3.3
FEV$_1$=2.3
PEFR=5.2
MEF40%=1.0
MEF40%(P)=0.7

POST-ALUPENT

FIVE-MINUTE POST-EXERCISE

VOLUME (L)

FLOW (L/SECOND)
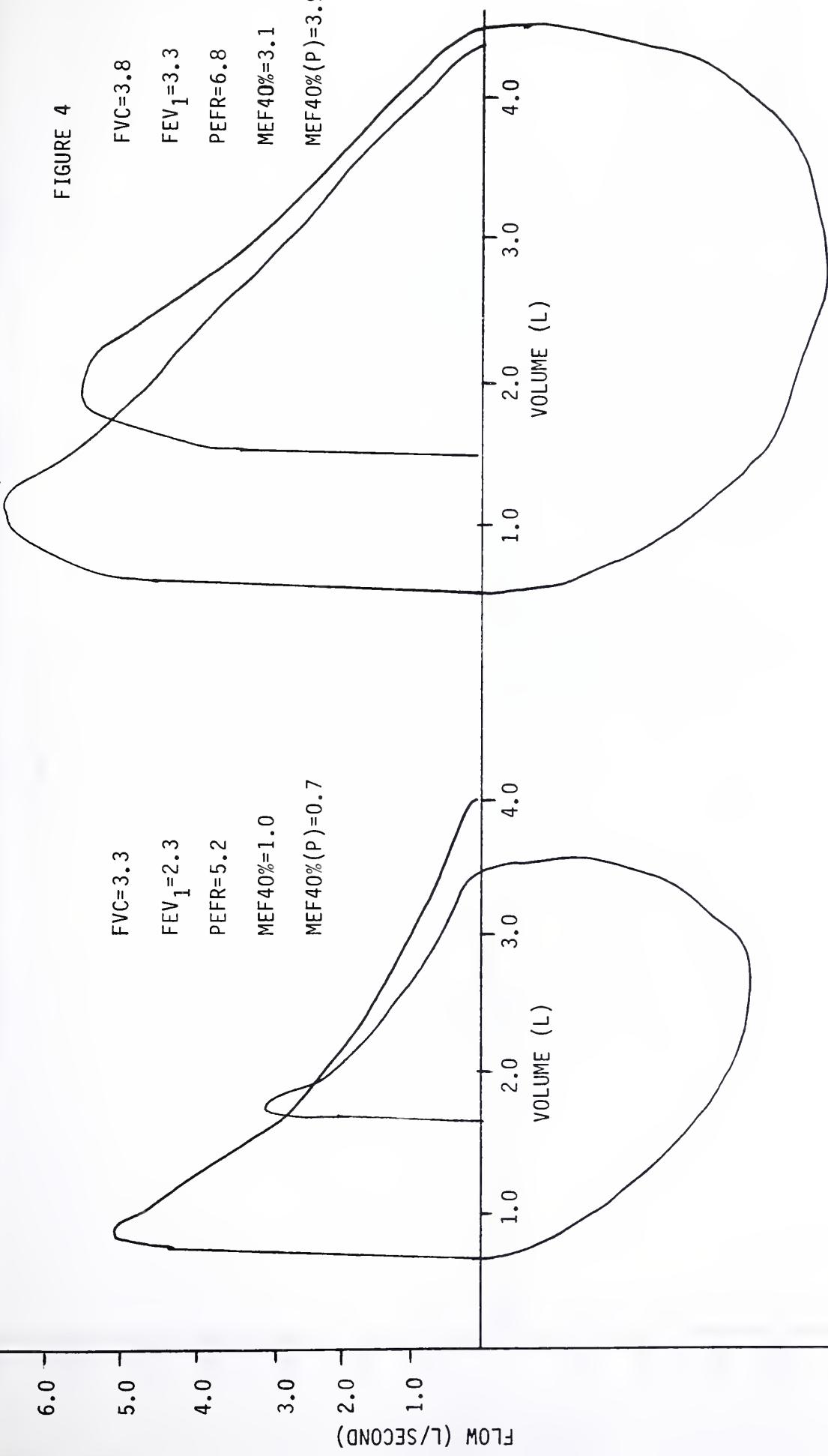
PULMONARY FUNCTION RESPONSE TO EXERCISE WITHOUT DRUGS

FIGURE 5

FVC (MEAN ± SEM)

ASTHMATICS

CONTROLS
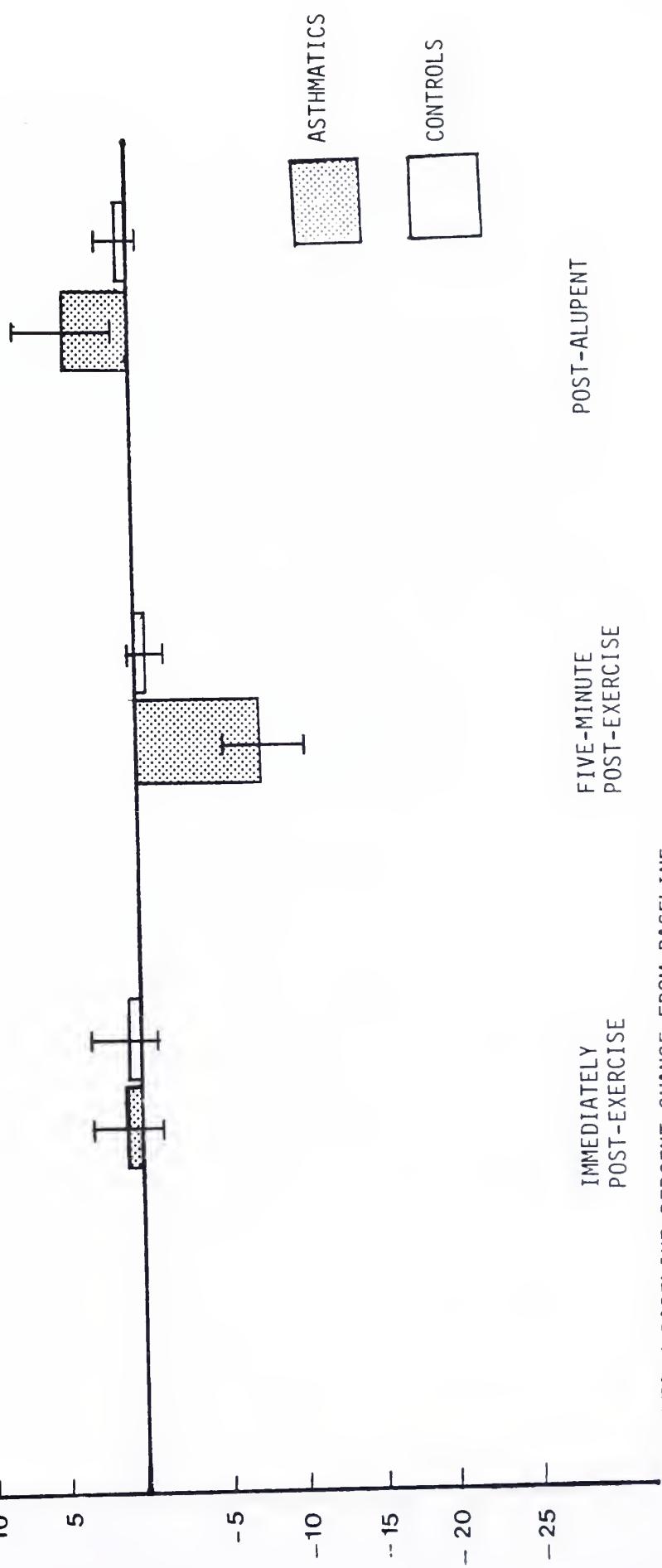
*DELTA % BASELINE=PERCENT CHANGE FROM BASELINE

IMMEDIATELY
POST-EXERCISE

FIVE-MINUTE
POST-EXERCISE

POST-ALUPENT

DELTA % BASELINE*

PULMONARY FUNCTION RESPONSE TO EXERCISE WITHOUT DRUGS   FIGURE 6
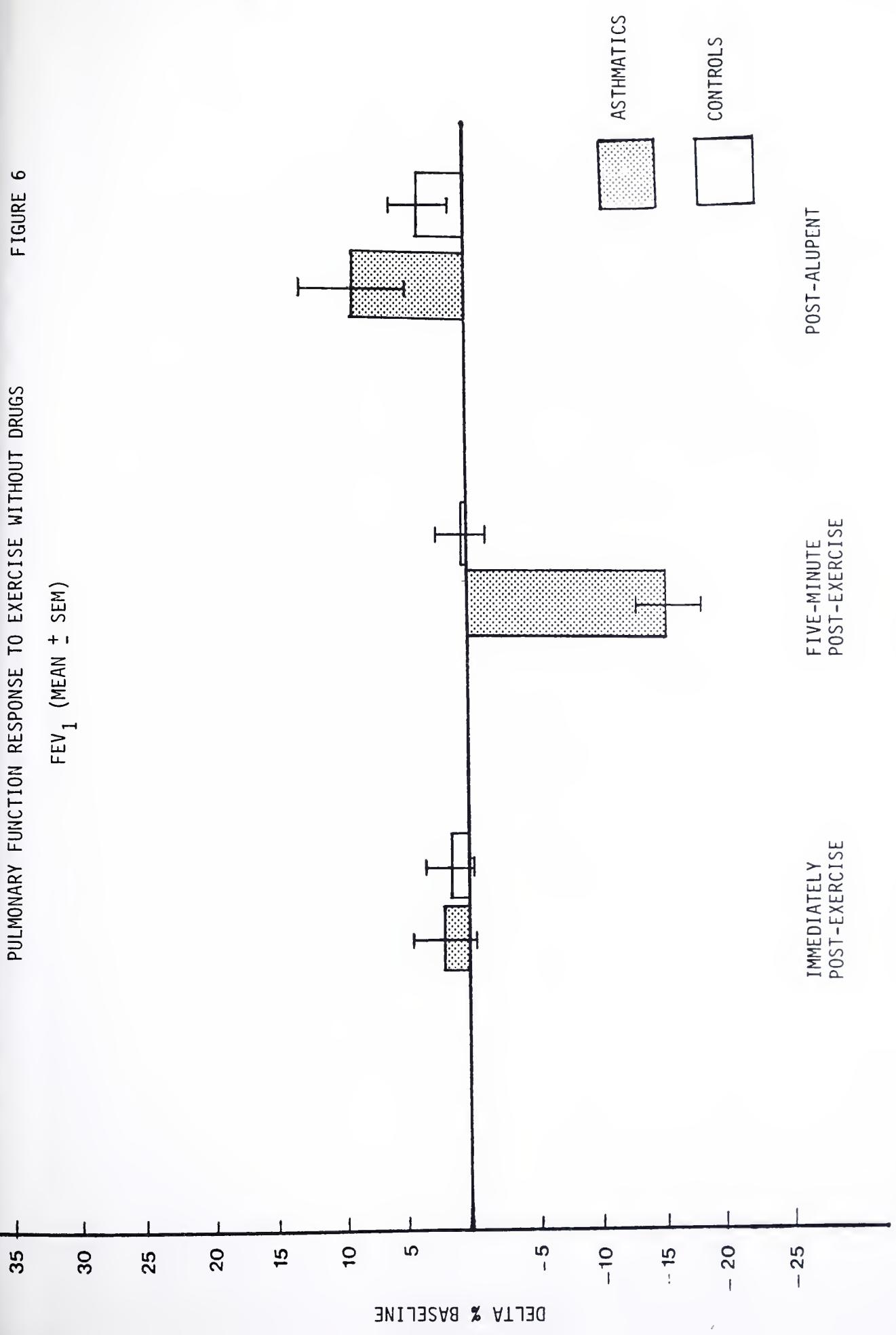
FEV$_1$ (MEAN $\pm$ SEM)

PULMONARY FUNCTION RESPONSE TO EXERCISE WITHOUT DRUGS

PEFR (MEAN ± SEM)
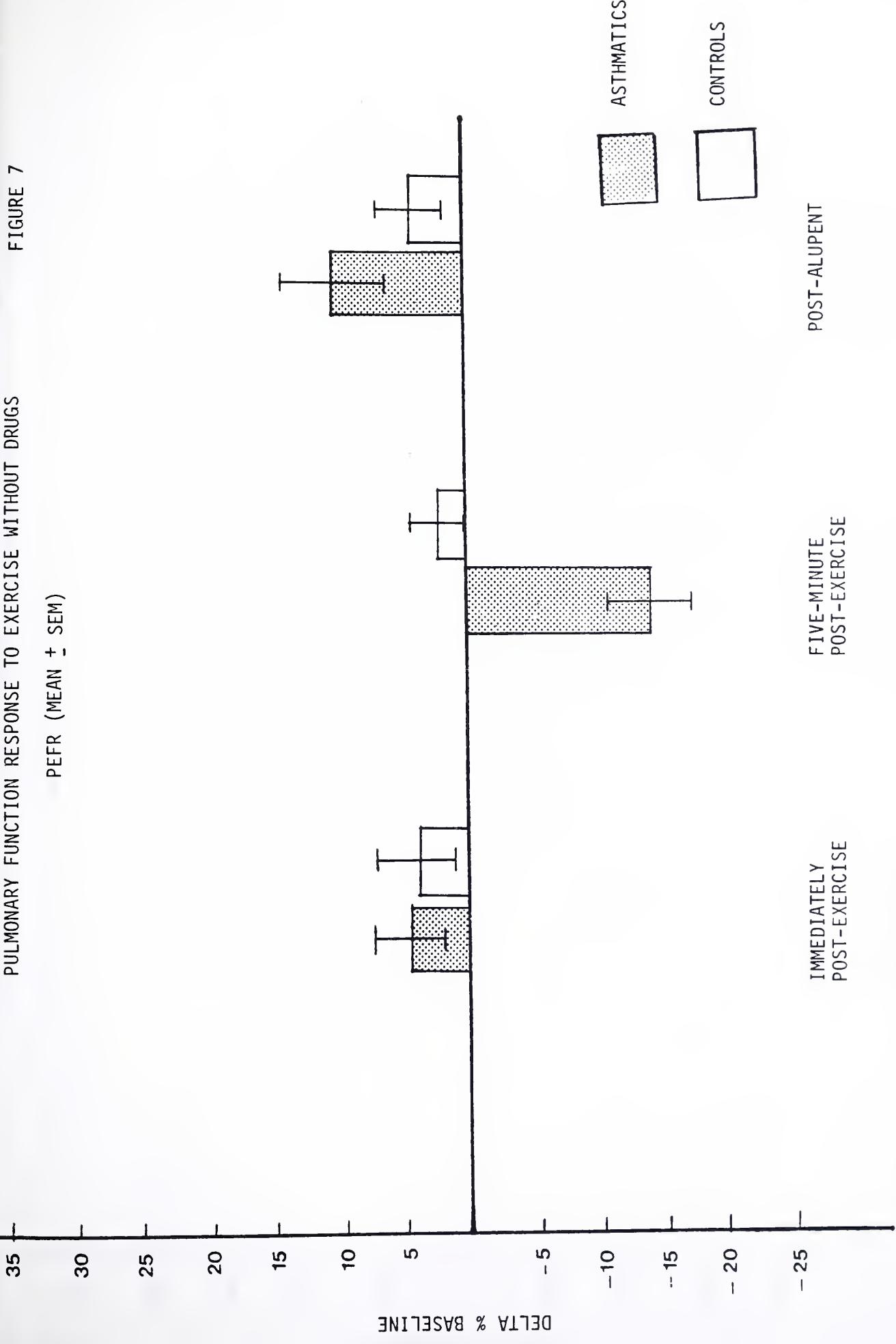
FIGURE 7

PULMONARY FUCTION RESPONSE TO EXERCISE WITHOUT DRUGS   FIGURE 8

MEF-40% (MEAN ± SEM)
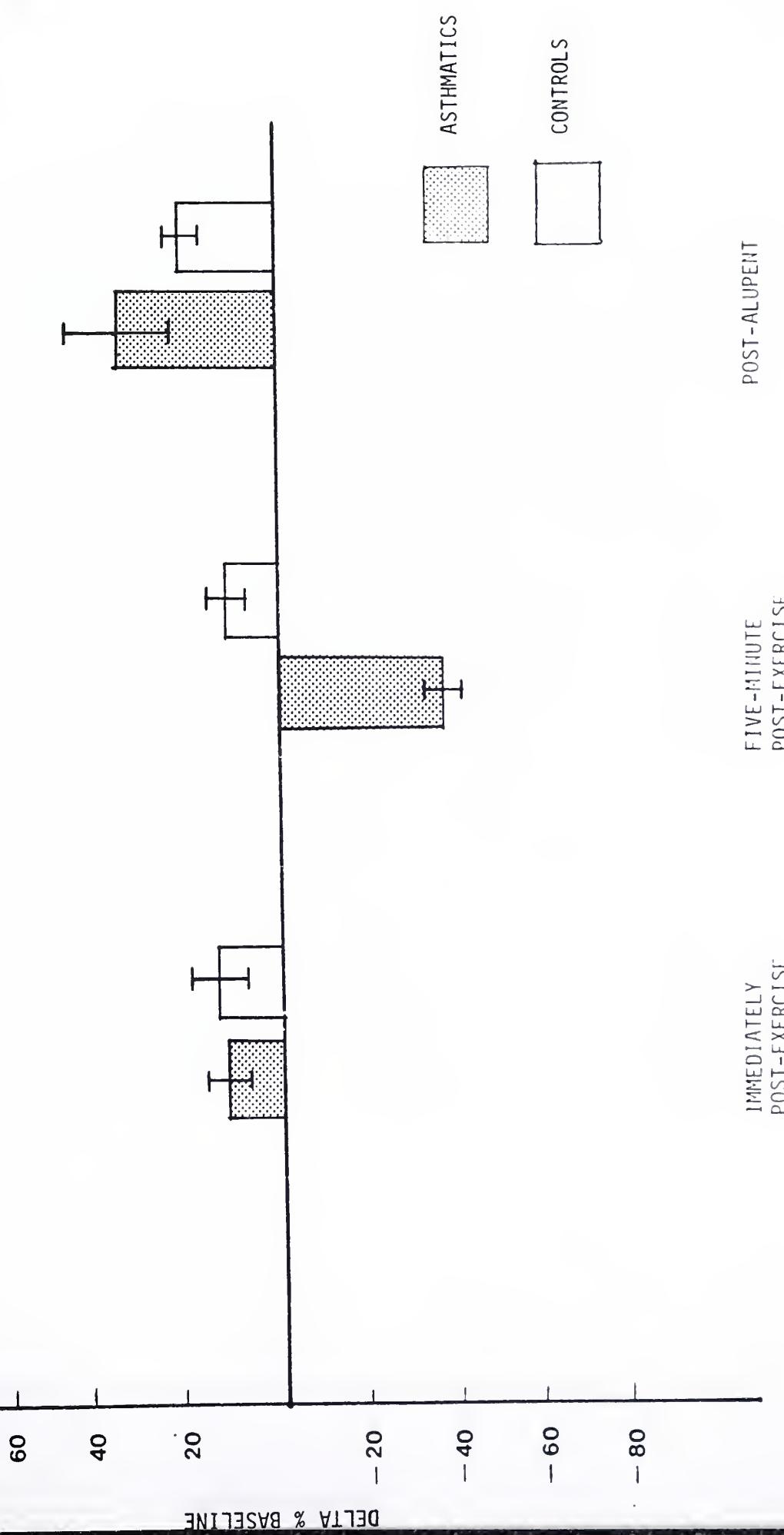
ASTHMATICS

CONTROLS

IMMEDIATELY
POST-EXERCISE

FIVE-MINUTE
POST-EXERCISE

POST-ALUPENT

DELTA % BASELINE

120
100
80
60
40
20
— 20
— 40
— 60
— 80

PULMONARY FUNCTION RESPONSE TO EXERCISE WITHOUT DRUGS

FIGURE 9
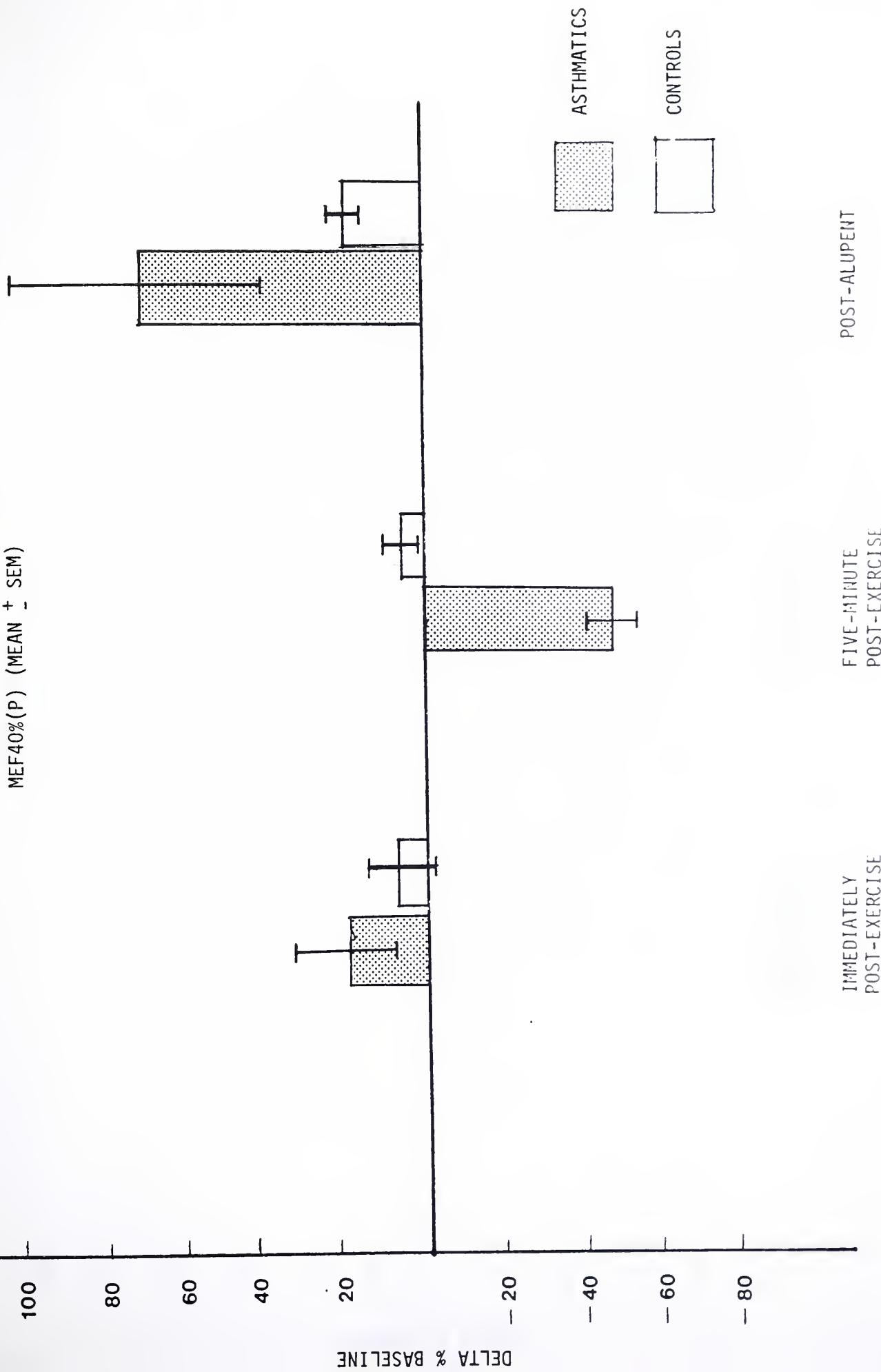
MEF40%(P) (MEAN ± SEM)

FIGURE 10

DELTA FVC: PLACEBO VS NO DRUG
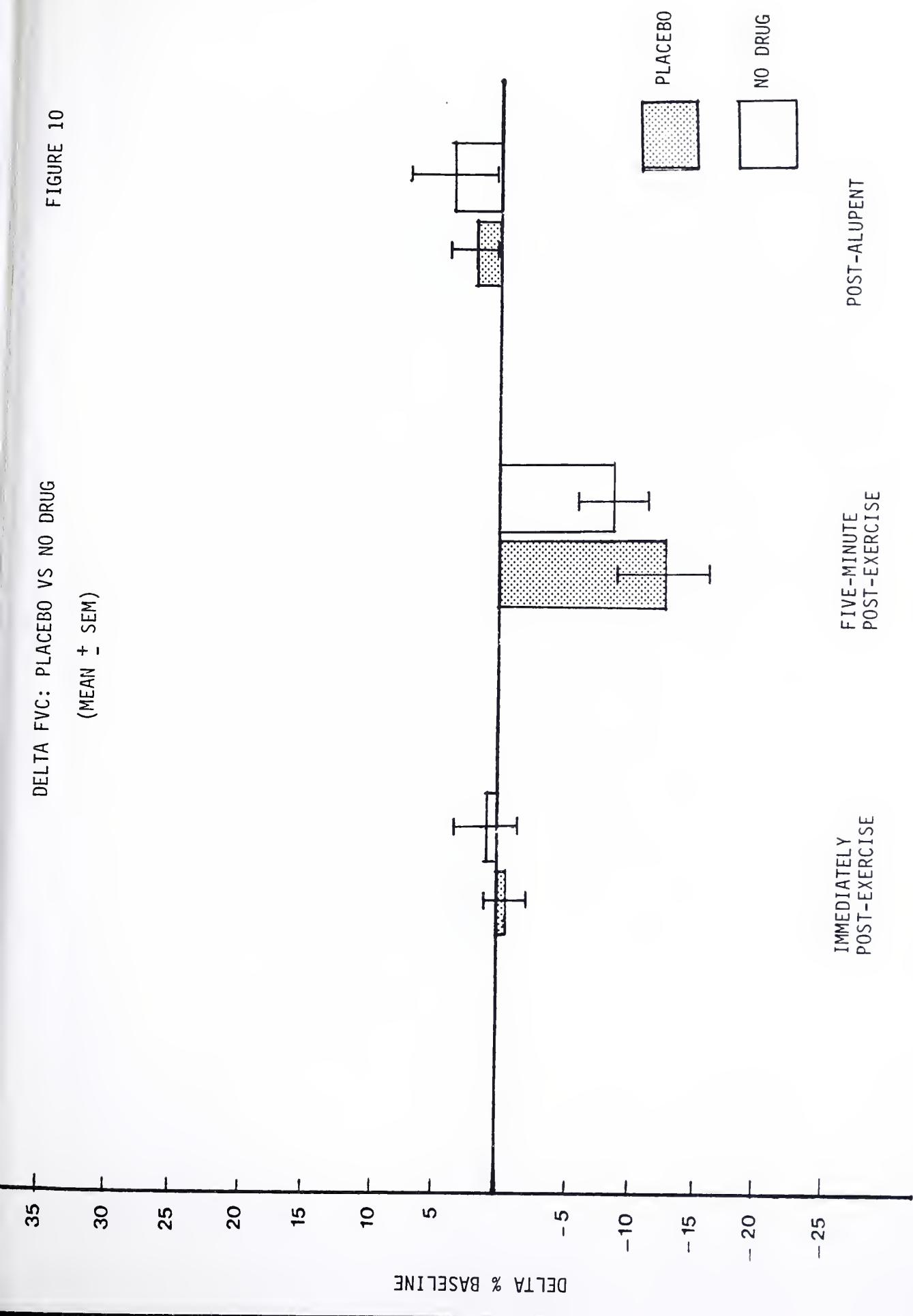
(MEAN ± SEM)

FIGURE 11

DELTA FEV$_1$: PLACEBO VS NO DRUG

(MEAN ± SEM)

DELTA % BASELINE
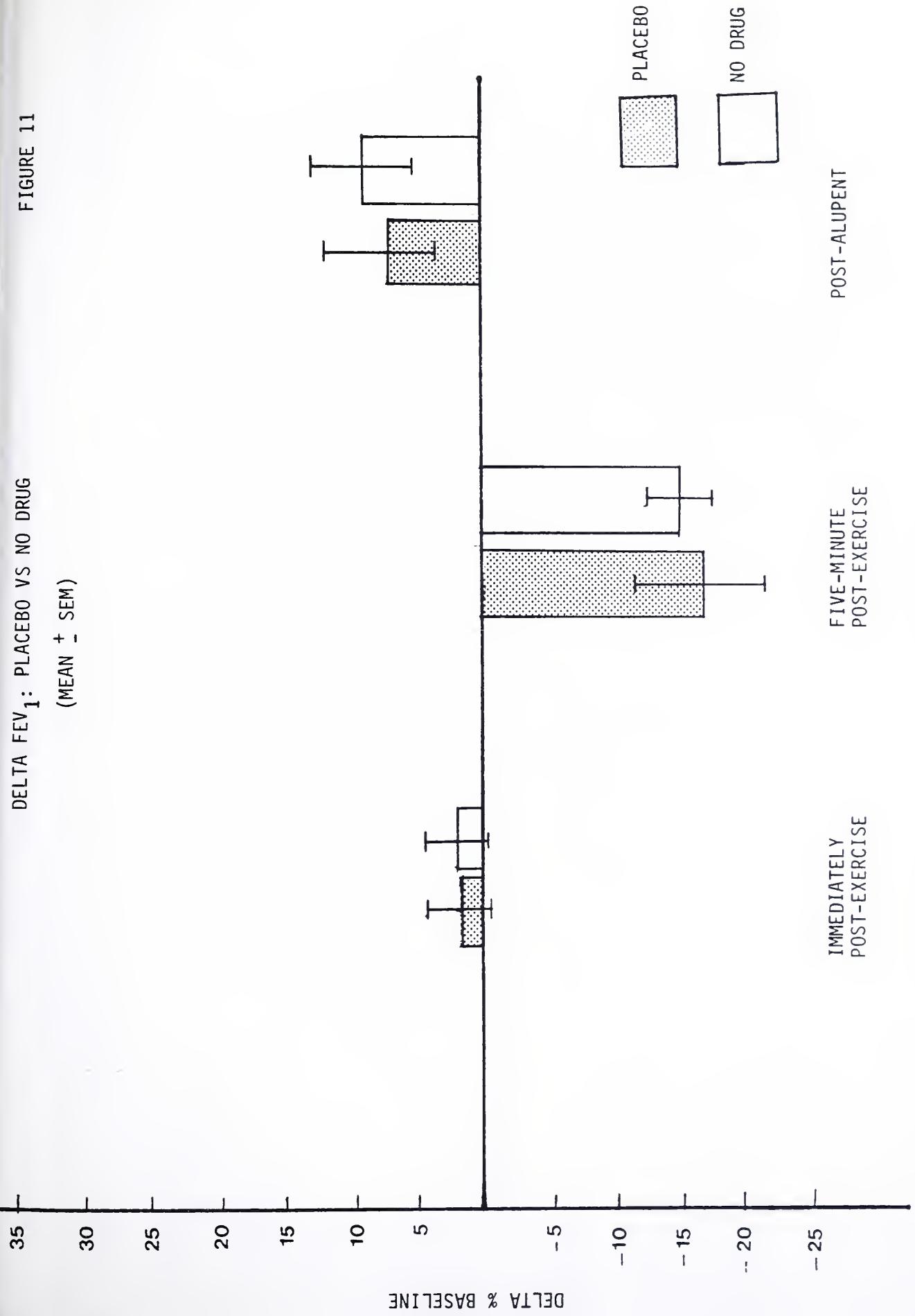
FIGURE 12

DELTA PEFR: PLACEBO VS NO DRUG
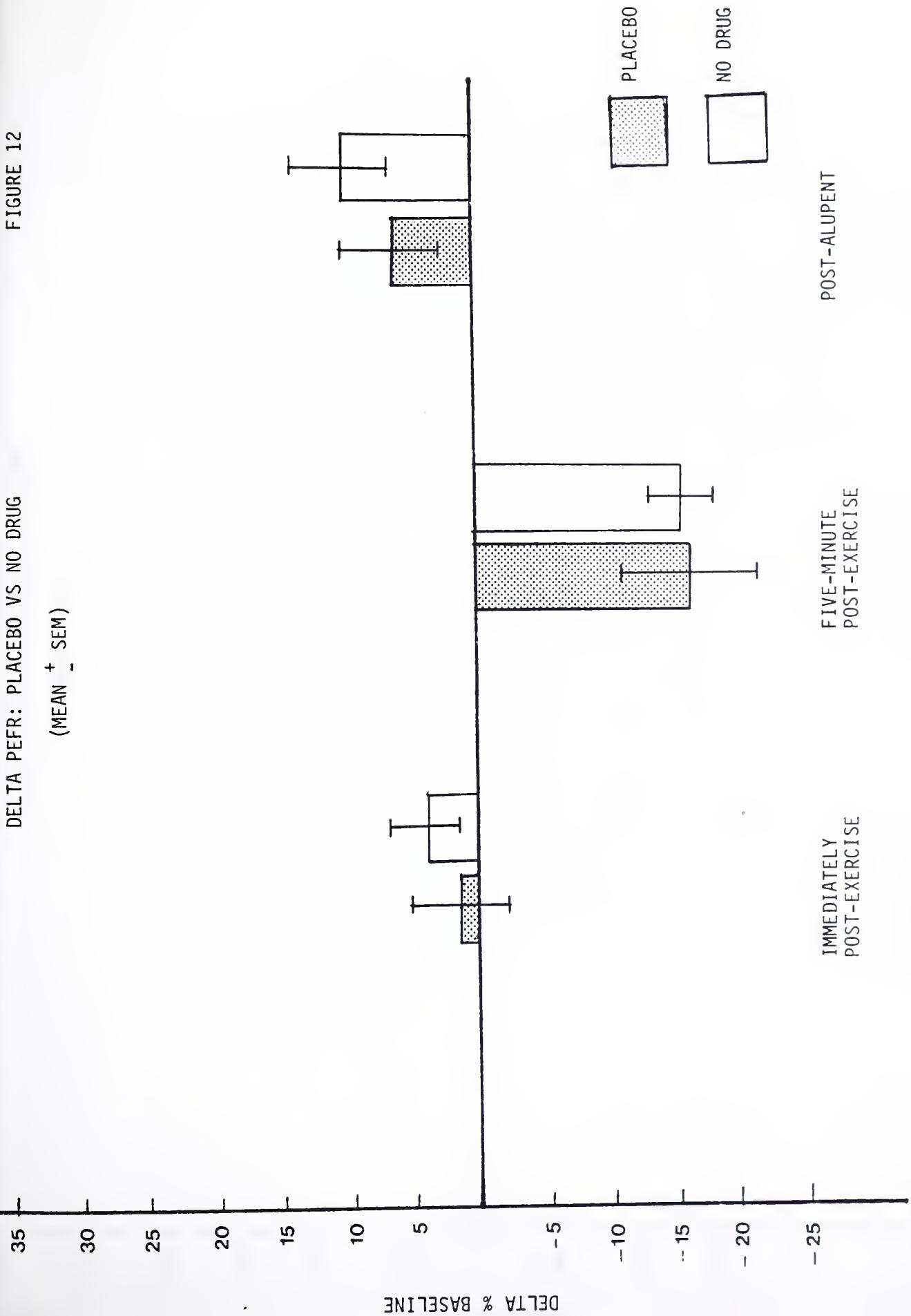
(MEAN $\pm$ SEM)

FIGURE 13

DELTA MEF40% : PLACEBO VS NO DRUG
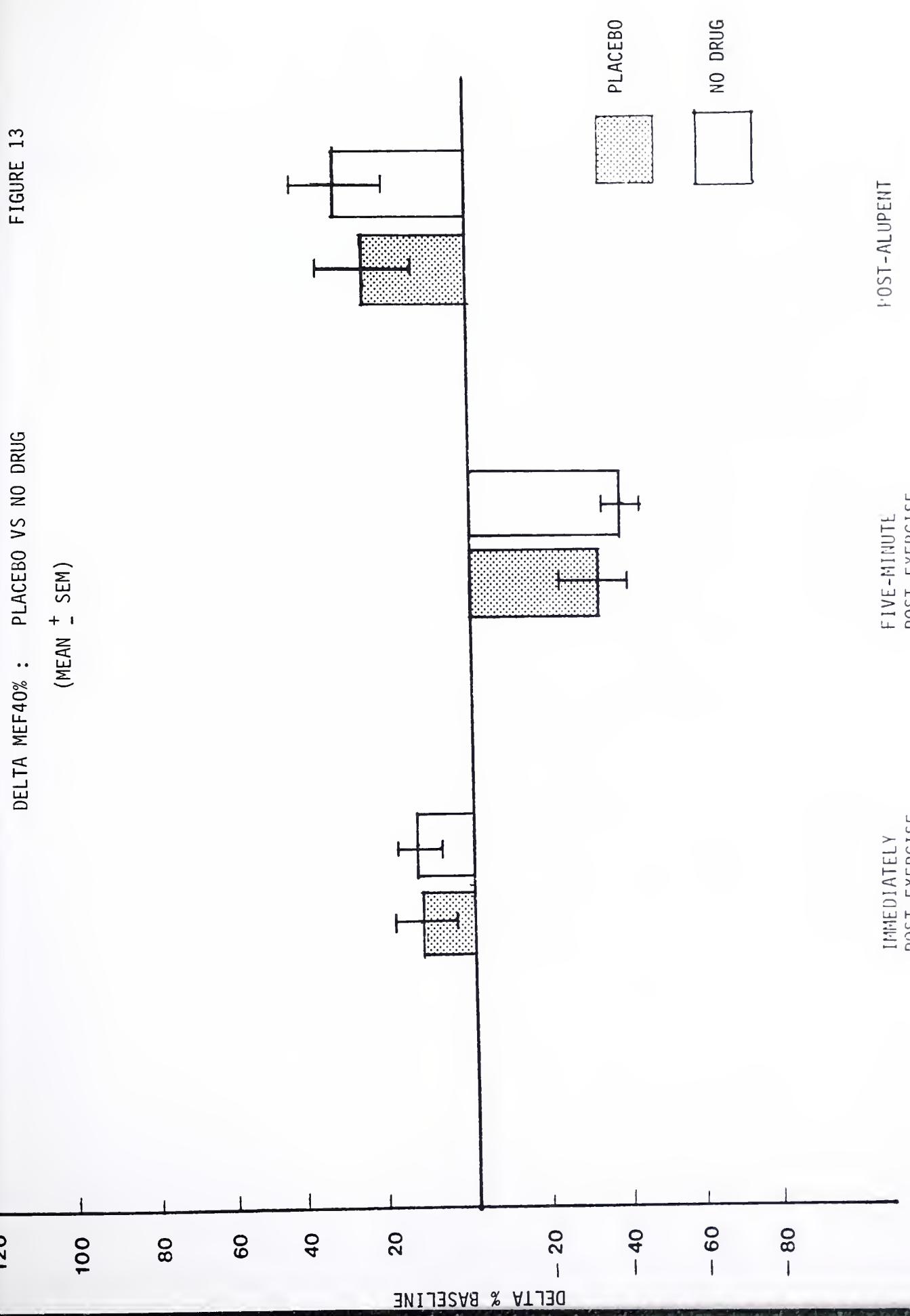
(MEAN ± SEM)

FIGURE 14

DELTA MEF40%(P): PLACEBO VS NO DRUG

(MEAN $\pm$ SEM)

PLACEBO

NO DRUG

POST-ALUPENT

FIVE-MINUTE
POST-EXERCISE

IMMEDIATELY
POST-EXERCISE

DELTA % BASELINE
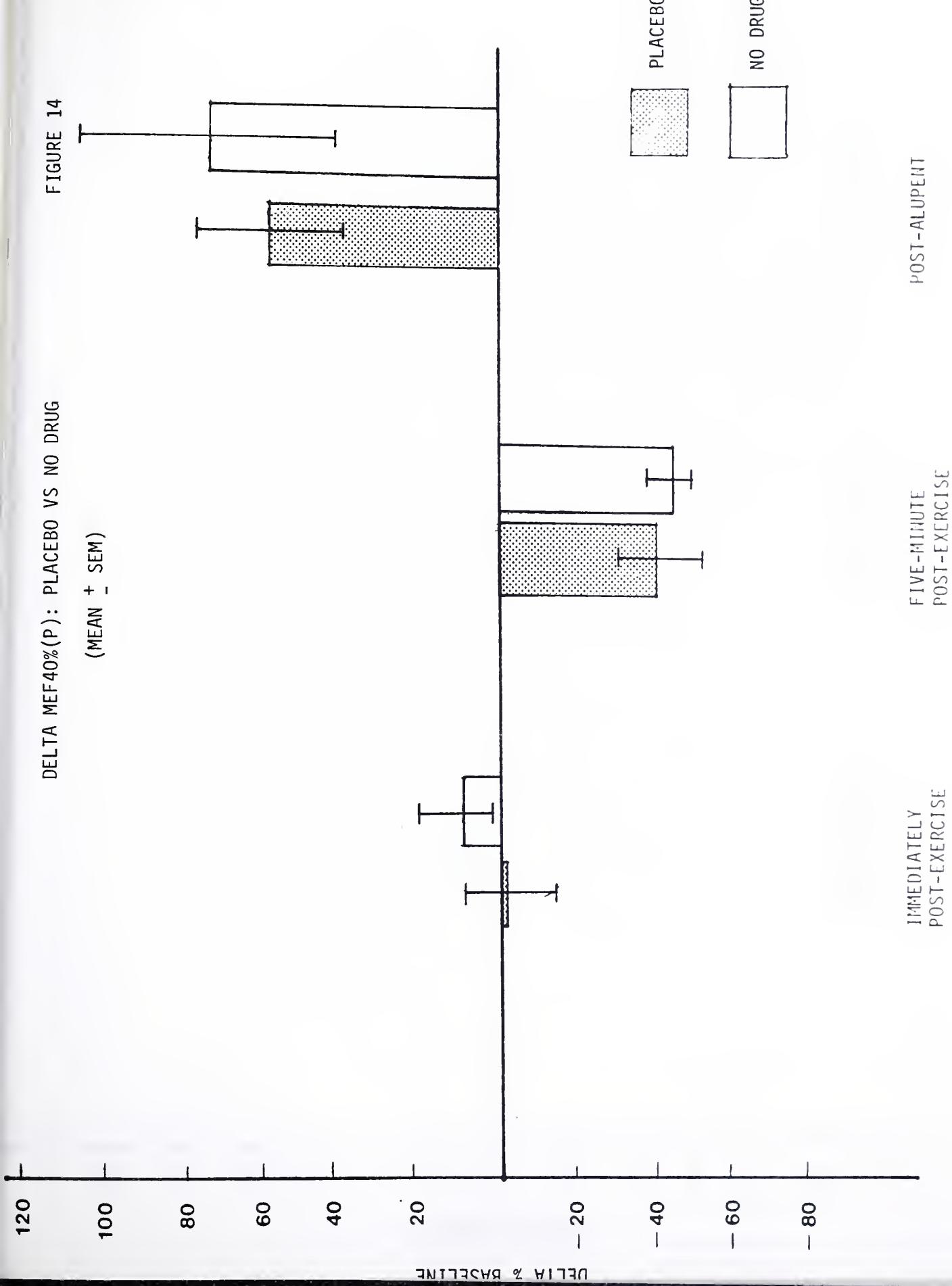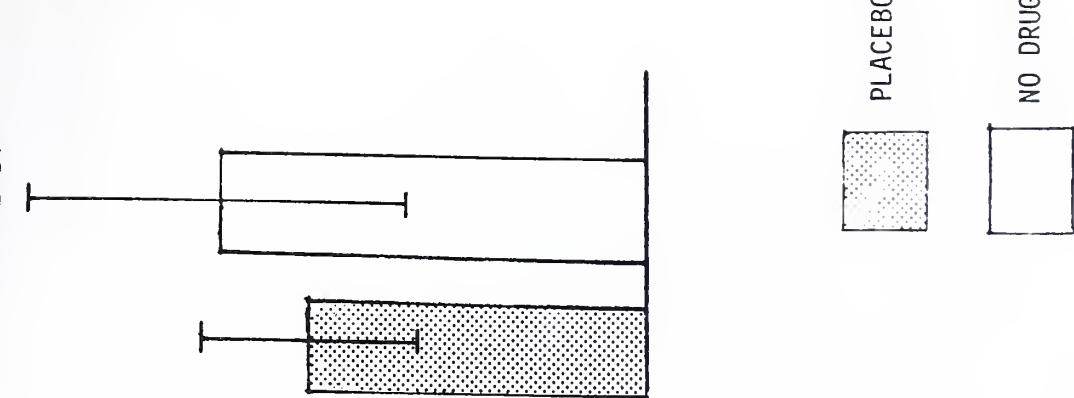
FIGURE 15

DELTA FVC: ASCORBIC ACID VS PLACEBO
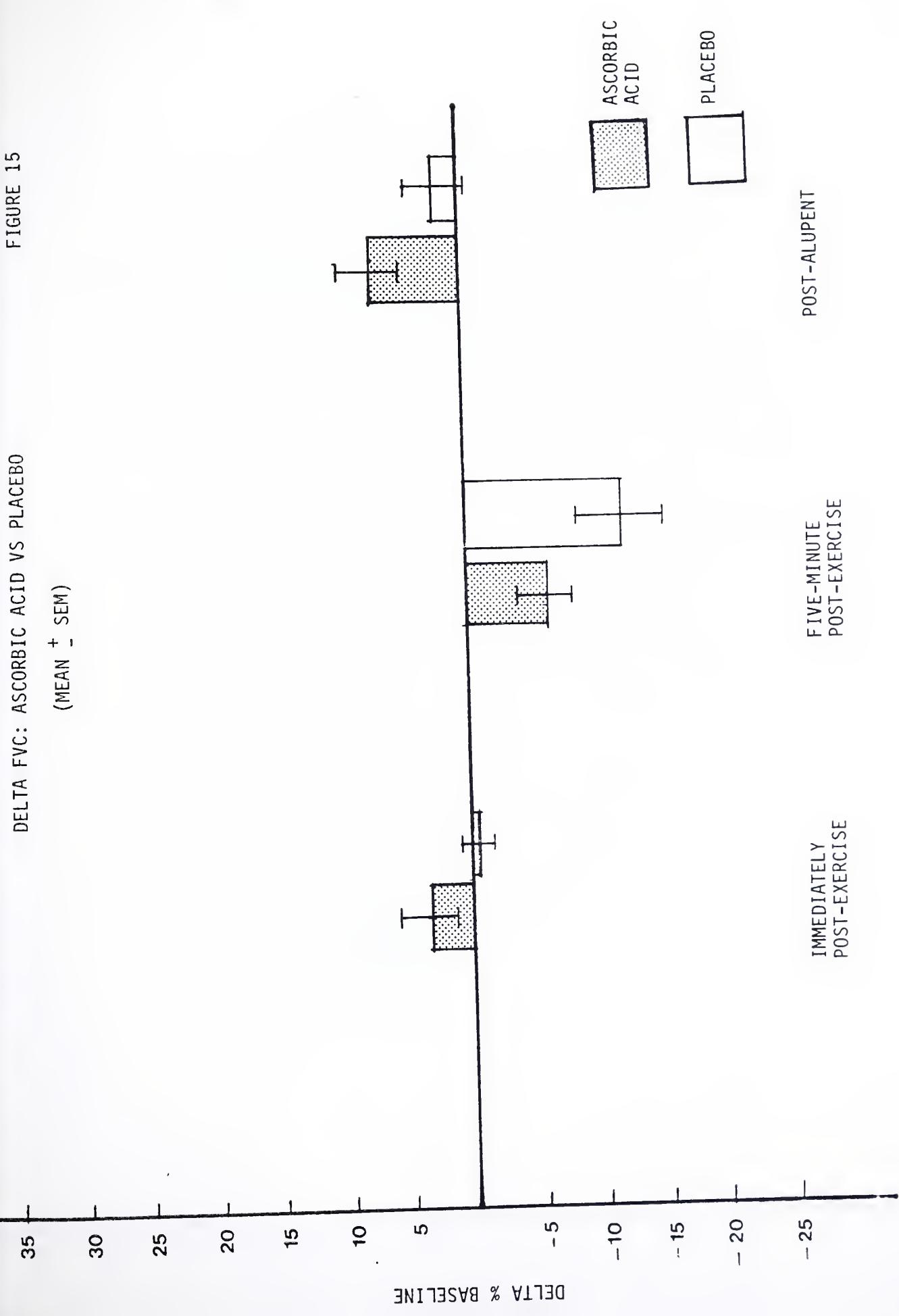
(MEAN ± SEM)

FIGURE 16

DELTA FEV₁: ASCORBIC ACID VS PLACEBO
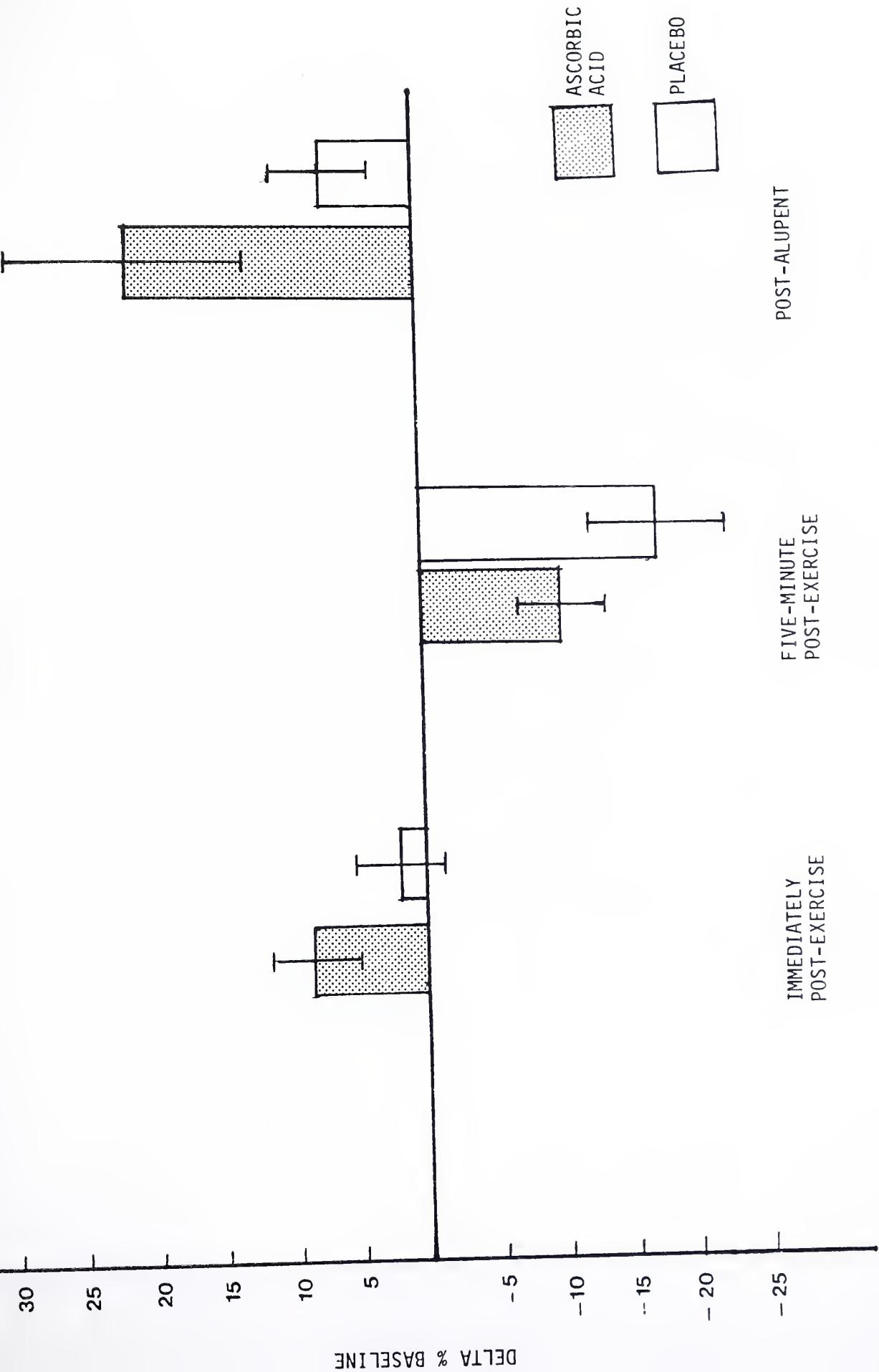
(MEAN ± SEM)

FIGURE 17

DELTA PEFR: ASCORBIC ACID VS PLACEBO
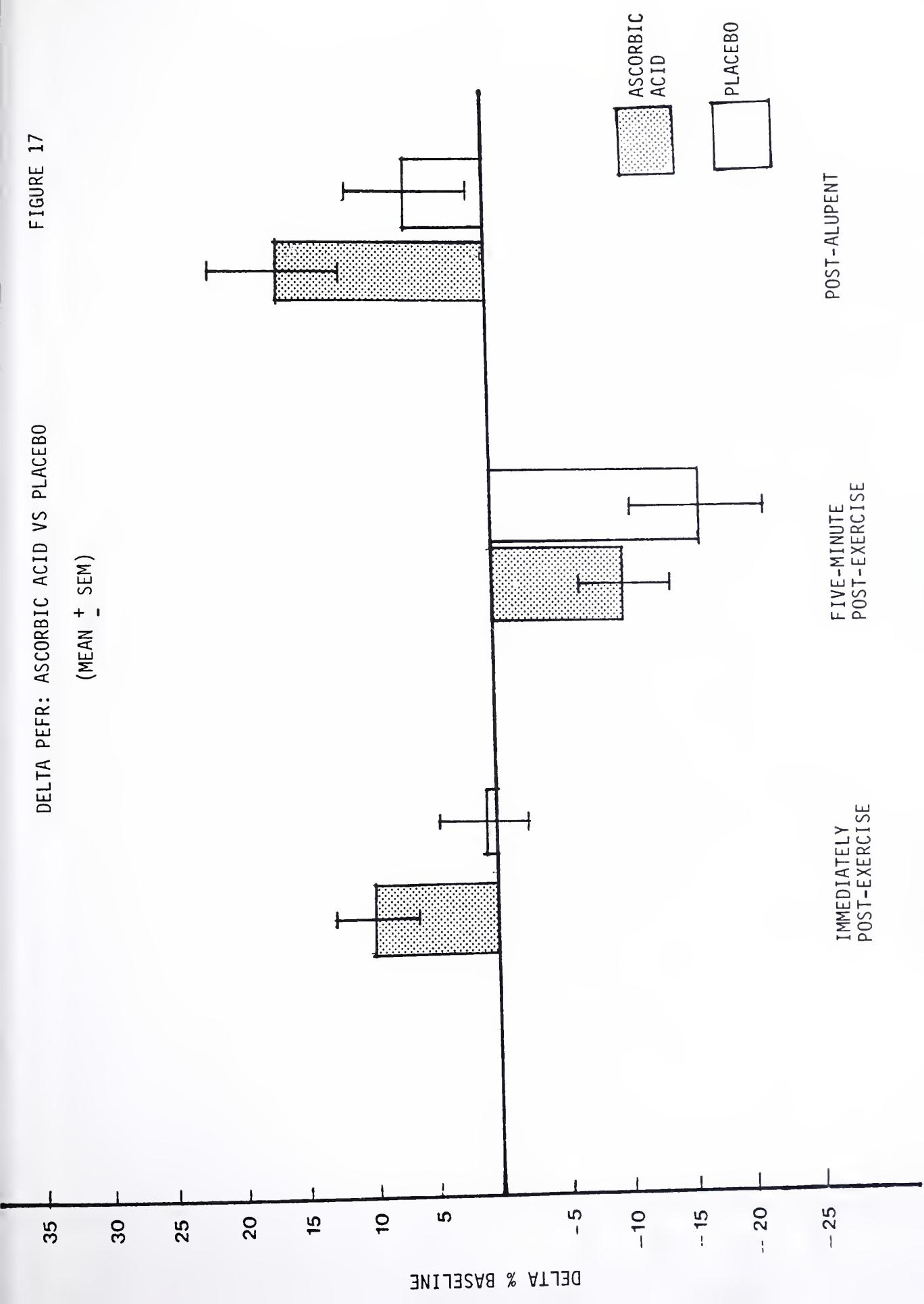
(MEAN ± SEM)

FIGURE 18

DELTA MEF40%: ASCORBIC ACID VS PLACEBO
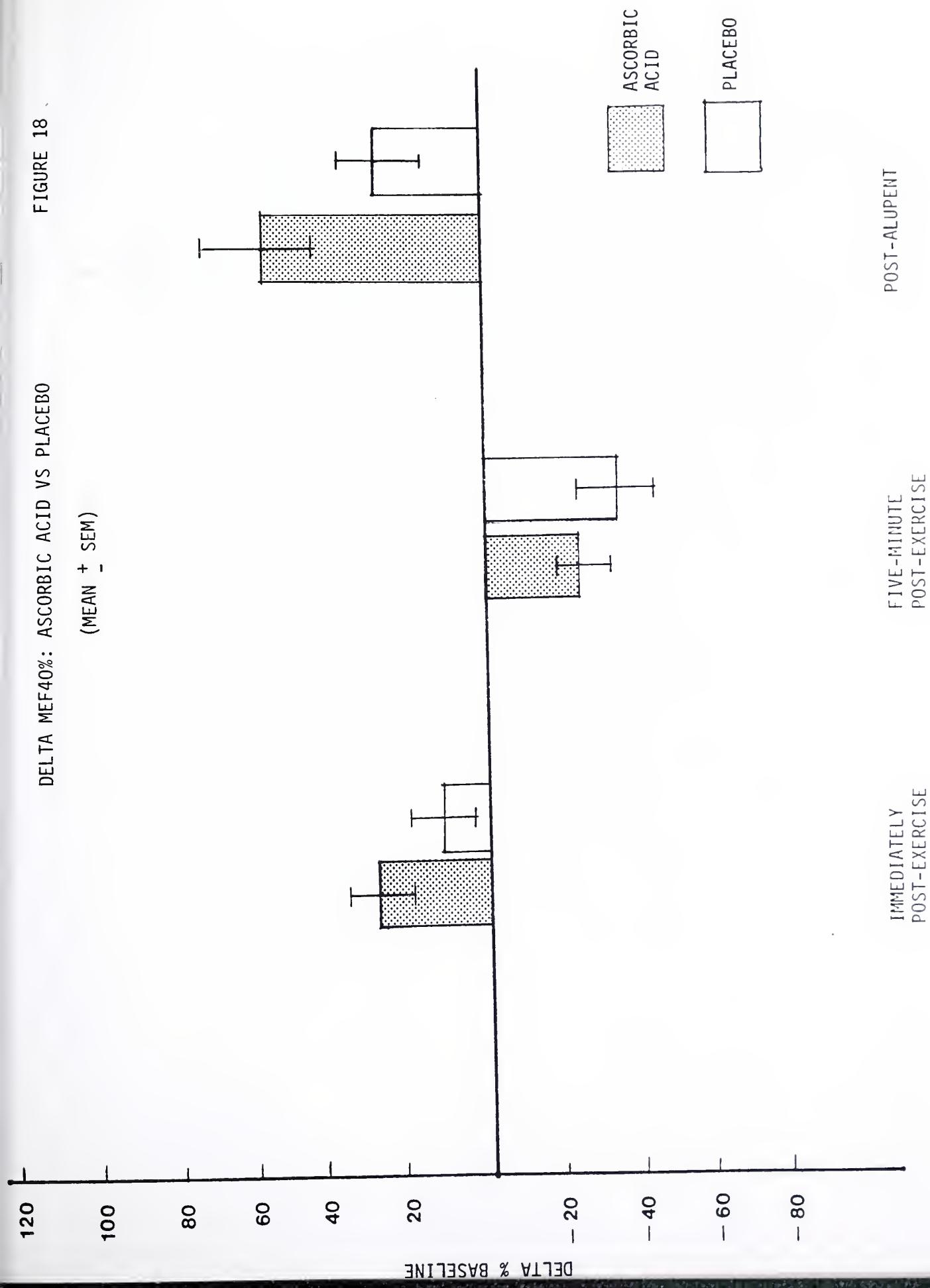
(MEAN ± SEM)

FIGURE 19

DELTA MEF40%(P): ASCORBIC ACID VS PLACEBO
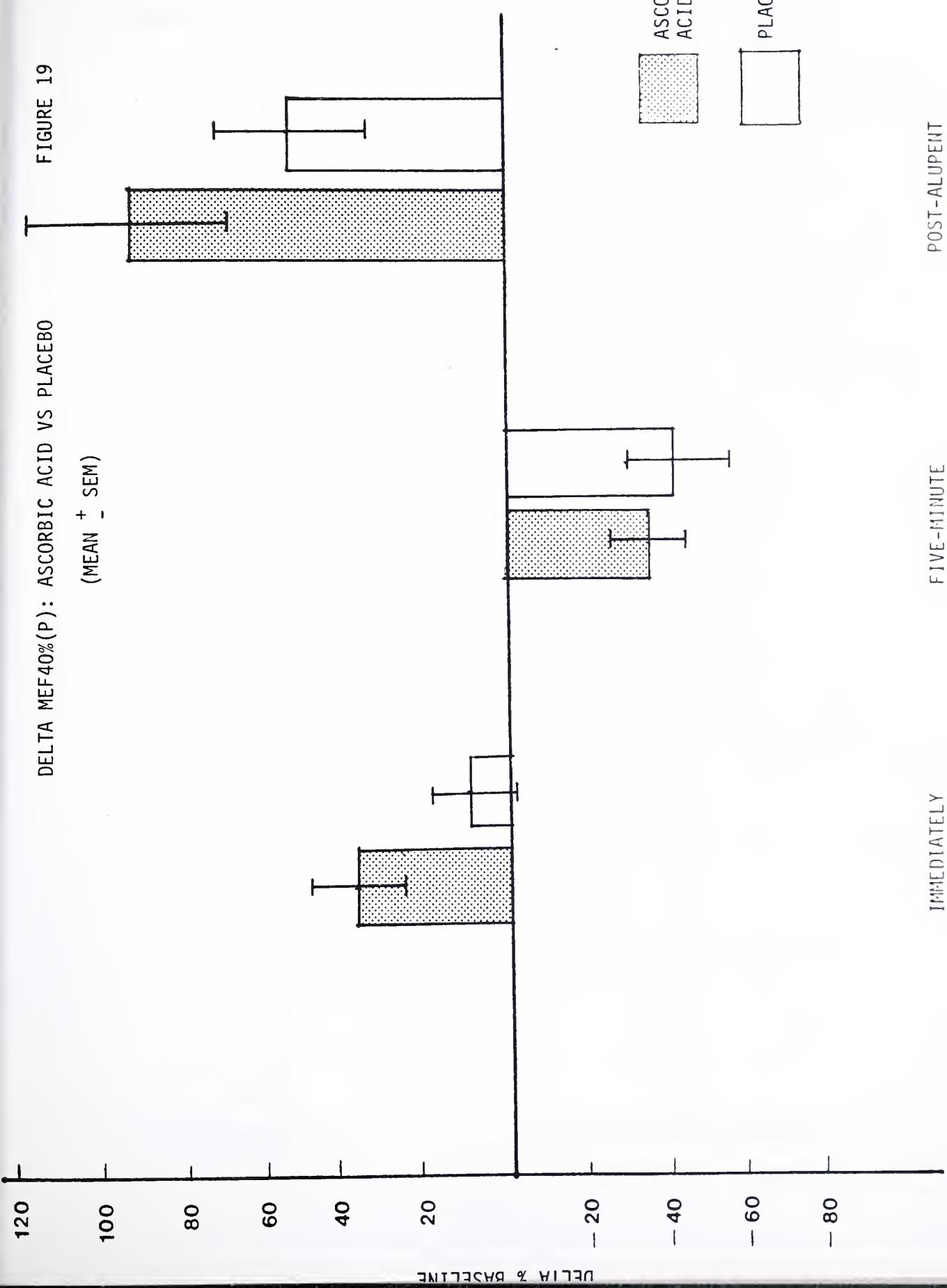
(MEAN $\pm$ SEM)

CHANGE IN FEV$_1$ AFTER EXERCISE IN ASTHMATIC SUBJECTS WITH PLACEBO
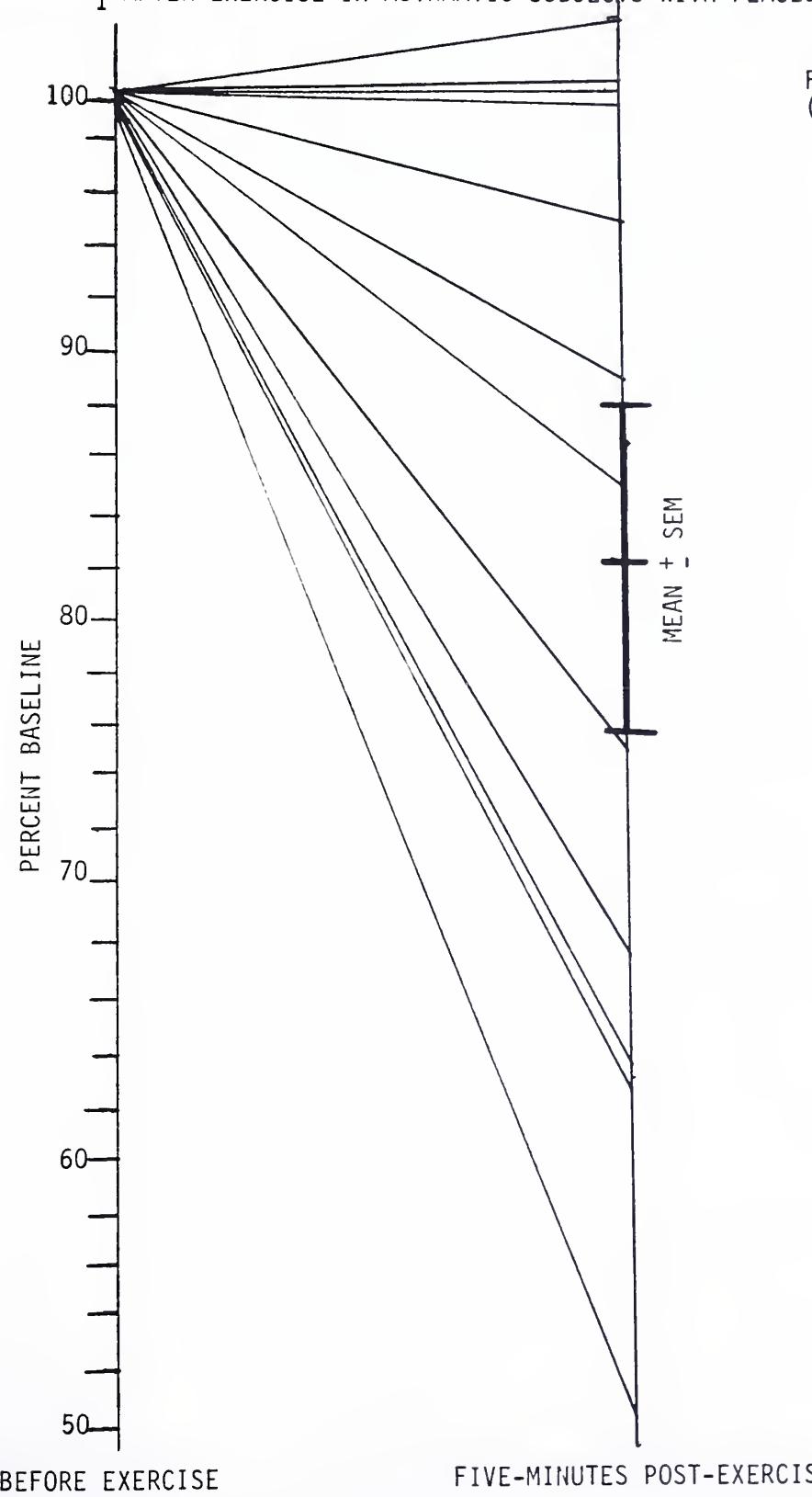
FIGURE 20
(PART 1)

CHANGE IN FEV$_1$ AFTER EXERCISE IN ASTHMATIC SUBJECTS WITH AA

FIGURE 20
(PART 2)

MEAN $\pm$ SEM

PERCENT BASELINE

100

90

80

70

60

50

BEFORE EXERCISE

FIVE-MINUTES POST-EXERCISE
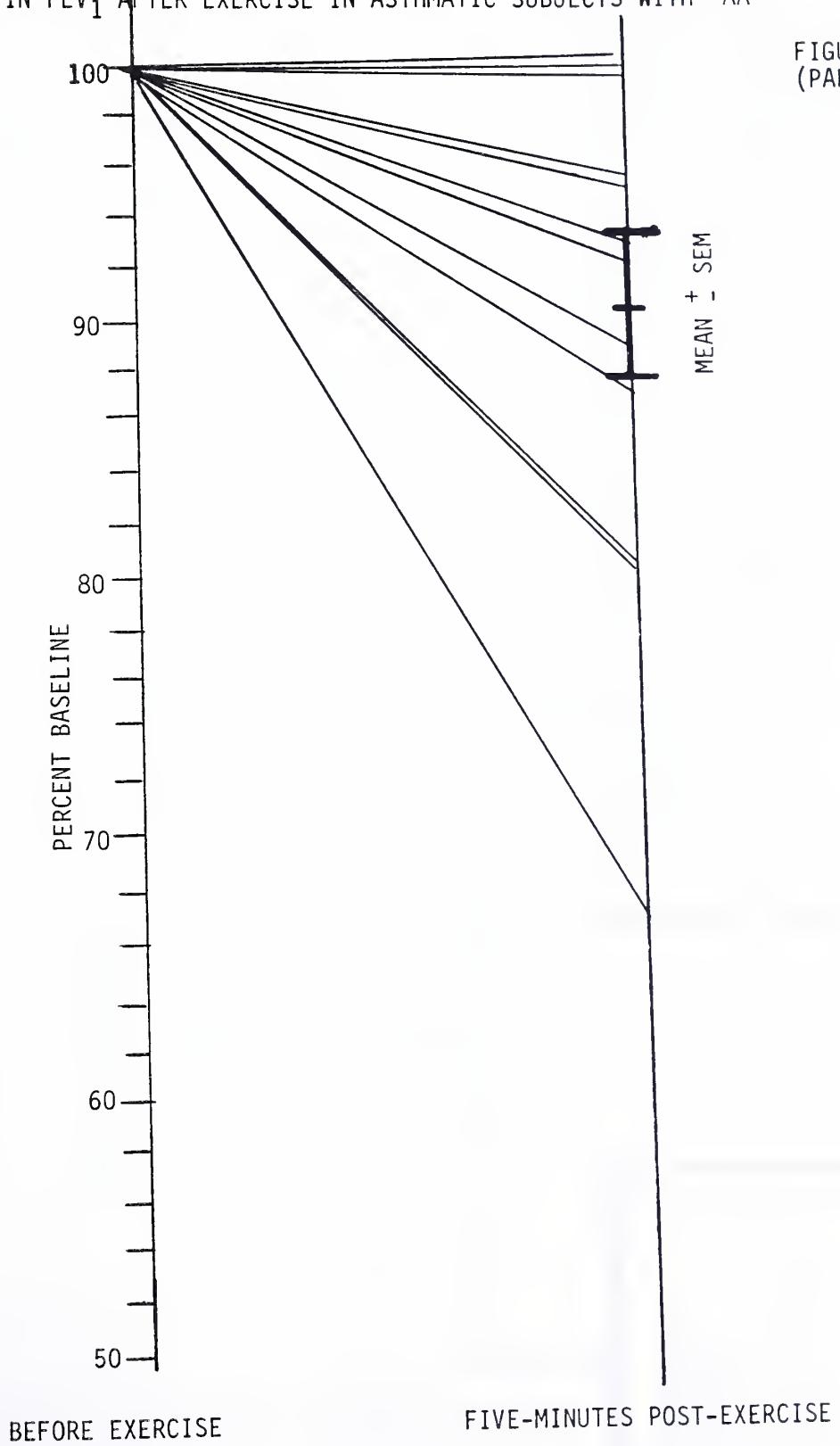
FIGURE 21

THEORIES FOR THE MECHANISM OF THE ATTENUATION OF EIB BY ASCORBIC ACID
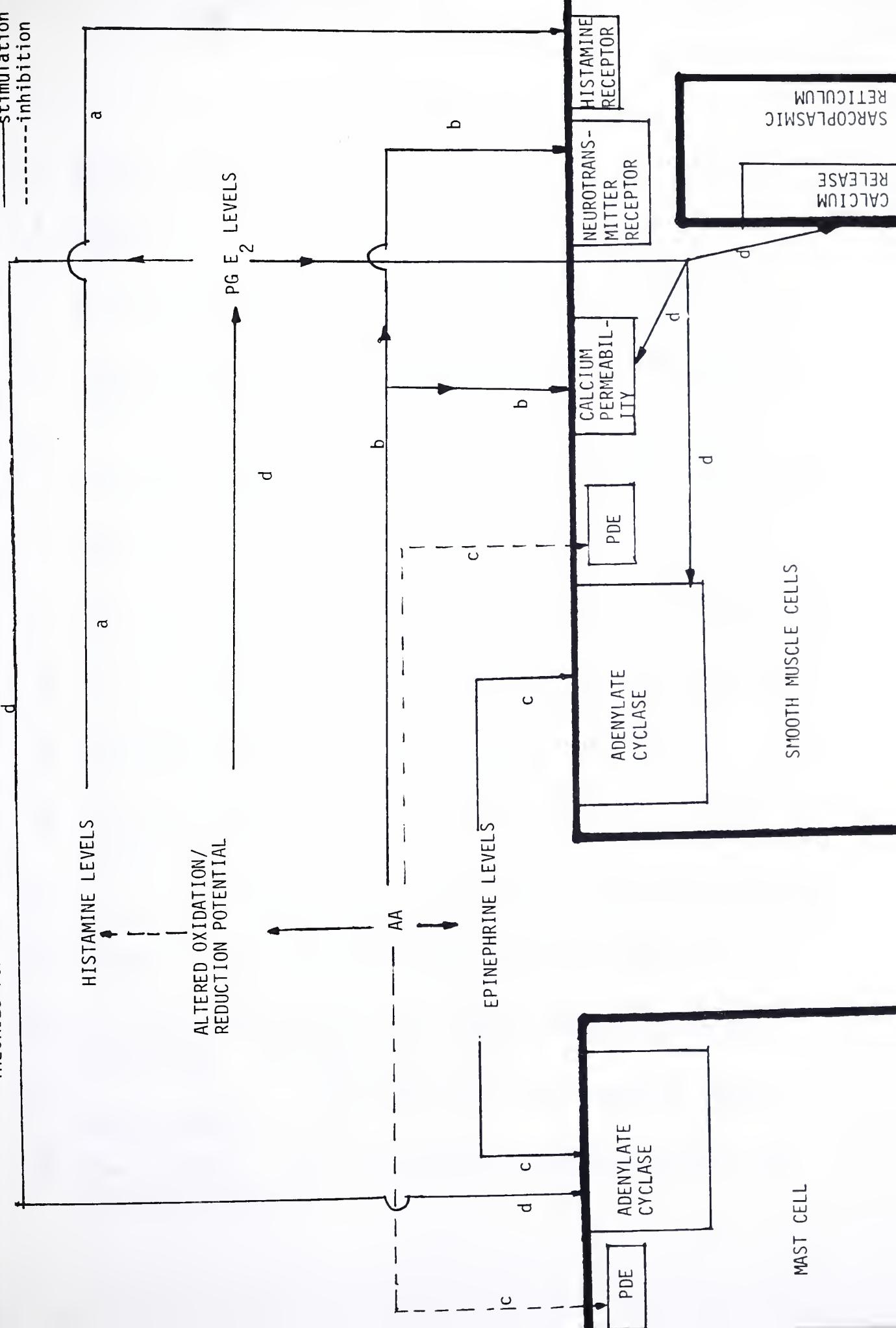
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